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5 to 8 July 2020

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ESHRE Journals, 5 Mill Yard, Childerley, Cambs CB23 8BA, United Kingdom
Telephone: +44 (0)1954 212404, editorial@humanreproduction.co.uk

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ORAL PRESENTATIONS

Monday, 06 July 2020

(*continued overleaf*)

17:00 - 18:00	Session 26: The day after. Fertility preservation and embryo transfer in patients with cancer diagnosis.	Parallel 5
17:00 - 18:00	Session 27: Frontiers in developmental biology	Parallel 6

Tuesday 07 July 2020

08:30 - 09:30	Session 28: Revisiting early embryo development	Parallel 1
08:30 - 09:30	Session 29: Building bridges towards harmonisation	Parallel 2
08:30 - 09:30	Session 30: Nurse or midwife led e-health care interventions.	Parallel 3
10:00 - 11:30	Session 31: Predictive algorithms in clinical embryology.	Parallel 1
10:00 - 11:30	Session 32: Which are the optimal ovarian stimulation protocol?	Parallel 2
10:00 - 11:30	Session 33: Predictors. Technology and processes improving outcomes in andrology.	Parallel 3
10:00 - 11:30	Session 34: Endometriosis - pathogenesis and diagnosis	Parallel 4
10:00 - 11:30	Session 35: Impact of ART on health outcomes of children	Parallel 5
10:00 - 11:30	Session 36: Covid-19 session	Parallel 6
11:45 - 12:45	Session 37: MHR symposium - fundamentals on making oocytes	Parallel 2
11:45 - 12:45	Session 38: Laboratory session - time-lapse in 2020.	Parallel 3
11:45 - 12:55	Session 39: Strategies to improve the outcomes of ovarian stimulation 2.	Parallel 4
14:00 - 14:45	Session 40: Global ART monitoring	Parallel 2
14:00 - 15:00	Session 41: ALMER exchange session - IVF laboratory automation	Parallel 3
14:00 - 15:00	Session 42: Stress and infertility - the chicken or the egg	Parallel 4
15:15 - 16:30	Session 43: ICSI in 2020	Parallel 1
15:15 - 16:30	Session 44: What are the optimal regimes for frozen embryo transfer?	Parallel 2
15:15 - 16:30	Session 45: Spermatogenesis subtle regulatory effectors	Parallel 3
15:15 - 16:30	Session 46: Reproductive (EPI)genetics 2.	Parallel 4
15:15 - 16:35	Session 47: Does emotional balance before being parents and after exist?.	Parallel 5
15:15 - 16:30	Session 48: Relating the relevance of biomarkers to infertility	Parallel 6
17:00 - 18:00	Session 49: Embryo metabolism and development	Parallel 1
17:00 - 18:00	Session 50: Androgen treatment in fertility management	Parallel 2
17:00 - 18:10	Session 51: RM: new diagnostic and therapeutic aspects	Parallel 3
17:00 - 18:00	Session 52: AI. A new tool to assess art outcomes and help patients?	Parallel 4
17:00 - 18:00	Session 53: Controversies in ART	Parallel 5
17:00 - 18:00	Session 54: Modern techniques promote variety in fertility nursing research	Parallel 6

Wednesday 08 July 2020

08:30 - 09:30	Session 55: Cochrane session - better evidence, better policies	Parallel 1
08:30 - 09:30	Session 56: Frontiers in andrology	Parallel 2
08:30 - 09:30	Session 57: Evidence-based surgical interventions	Parallel 3
08:30 - 09:30	Session 58: Improving sperm crysopreservation outcomes.	Parallel 4

10:00 - 11:45	Session 59: New morphokinetic insights of embryo development	Parallel 1
10:00 - 11:45	Session 60: Long term health, obstetrics and neonatal outcomes relating to infertility treatment	Parallel 2
09:50 - 11:55	Session 61: Understanding spermatogenesis beyond histology	Parallel 3
10:00 - 11:45	Session 62: Pathophysiologic aspects of implantation	Parallel 4
10:00 - 11:45	Session 63: Protecting gamete quality	Parallel 5
10:00 - 11:45	Session 64: Prospective carrier screening of ART couples	Parallel 6
12:00 - 13:00	Session 65: Biomarkers of failed pregnancy	Parallel 1
12:00 - 13:00	Session 66: Synthetic embryology: myth or reality?	Parallel 2
12:00 - 13:00	Session 67: COVID-19 - Psychosocial impact of delayed treatment	Parallel 3
12:00 - 13:00	Session 68: Genetic determinants of embryo quality	Parallel 4
14:00 - 15:15	Session 69: Biomarkers of developmental competence	Parallel 1
14:00 - 15:15	Session 70: Ovarian stimulation strategies in IVF and IUI	Parallel 2
14:00 - 15:15	Session 71: About how sperm quality and male infertility relate to genetics	Parallel 3
14:00 - 15:15	Session 72: Pregnancy loss: what to consider	Parallel 4
14:00 - 15:15	Session 73: Endometriosis and ART	Parallel 5
14:00 - 15:15	Session 74: Oocyte and embryo evaluation	Parallel 6

- **INVITED SESSIONS**
- **SELECTED ORAL COMMUNICATION SESSIONS**

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INVITED SESSION
SESSION 01: KEYNOTE SESSION
06 July 2020 Parallel 1 08:30 - 09:30

O-001 Harnessing the power of data: Application of data science in reproductive medicine

S. Nelson[1]

[1]*University of Glasgow, Royal Fertility Clinic, Glasgow, United Kingdom*

O-002 Association of phthalates, parabens and phenols found in personal care products with pubertal timing in girls and boys

K.G. Harley[1], **K. Berger**[1], **K. Kogut**[1], **K. Parra**[2], **R. Lustig**[3], **L. Greenspan**[4], **A. Calafat**[5], **B. Eskenazi**[1]

[1]*UC Berkeley, School of Public Health, Berkeley- CA, U.S.A.*
[2]*University of Arizona, Mel and Enid Zuckerman College of Public Health, Tucson- AZ, U.S.A.*
[3]*University of California- San Francisco, Department of Pediatrics, San Francisco, U.S.A.*
[4]*Kaiser Permanente, Department of Pediatrics, San Francisco, U.S.A.*
[5]*Centers for Disease Control and Prevention, National Center for Environmental Health, Atlanta- GA, U.S.A.*

Background: Certain chemicals in cosmetics and personal care products, including low molecular weight phthalates, parabens, and phenols, have demonstrated endocrine disrupting properties. These chemicals have been associated with altered pubertal timing in animal studies, but few human studies exist.
Methods: Data were from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) longitudinal cohort study that followed 338 children in the Salinas Valley, California from before birth to adolescence. Pregnant women were enrolled in 1999-2000. Mothers were mostly Latina, living below the federal poverty threshold, and had not completed high school. We measured concentrations of three phthalate metabolites (monoethyl phthalate [MEP], mono-n-butyl phthalate, and mono-isobutyl phthalate), methyl and propyl paraben, and four other phenols (triclosan, benzophenone-3, and 2,4- and 2,5-dichlorophenol) in urine collected from mothers during pregnancy and from children when they were 9 years of age. Pubertal timing was assessed among 179 girls and 159 boys every 9 months between ages 9 and 13 using clinical Tanner staging. Accelerated failure time models were used to obtain mean shifts of pubertal timing associated with concentrations of prenatal and peripubertal biomarkers.
Results: In girls, we observed associations of earlier onset of pubic hair development with increased prenatal urinary MEP concentrations and earlier menarche with increased prenatal triclosan and 2,4-dichlorophenol concentrations. With peripubertal biomarkers, we observed earlier breast development, pubic hair development, and menarche with increased methyl paraben urinary concentrations; earlier menarche with propyl paraben concentrations; and later pubic hair development with 2,5-dichlorophenol concentrations. In boys, we observed no associations with prenatal urinary biomarker concentrations, and only one association – of earlier pubic hair development with propyl paraben – with peripubertal concentrations.
Conclusions: We found associations of altered pubertal timing with early life exposure to chemicals in personal care products in girls, but little evidence in boys. This study contributes to a growing literature that suggests that exposure to certain endocrine disrupting chemicals may impact timing of puberty in children.

SELECTED ORAL COMMUNICATIONS
SESSION 02: BLASTOCYST TRANSFER AND FREEZING
06 July 2020 Parallel 1 10:00 - 11:30

O-003 The effect of laser assisted hatching on vitrified-warmed blastocysts: an interim analysis of a multicentric randomized controlled trial

A. Alteri[1], **C. Guarneri**[2], **L. Corti**[1], **L. Restelli**[2], **M. Reschini**[2], **N. Barberis**[1], **E. Papaleo**[1], **A. Paffoni**[3], **P. Viganò**[4], **E. Somigliana**[2]

[1]*IRCCS San Raffaele Scientific Institute, Obstetrics and Gynecology Department, Milan, Italy ;*
[2]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Obstetrics and Gynaecology Department, Milan, Italy ;*
[3]*ASST Lariana, Infertility Unit, Cantù, Italy ;*
[4]*IRCCS San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Milan, Italy*

Study question: Does assisted hatching of vitrified/warmed blastocysts improve clinical outcomes?
Summary answer: No significant difference was found in clinical pregnancy rate (CPR).
What is known already: Recently, the Human Fertilization and Embryology (HFEA) declared that the assisted hatching (AH) is an unproven add-on intervention and the guidelines of the National Institute for Clinical Excellence (NICE) do not recommend the use of AH to improve pregnancy rates. Of note, available data are mainly focused on the effect of AH in fresh-cleavage stage embryo transfers while data regarding the effect of laser AH (LAH) on blastocysts, especially after cryopreservation, are inconclusive since they are based on studies with small sample sizes and may hide a type II error.
Study design, size, duration: A multicentric prospective comparative study with a parallel randomized controlled design. The sample size (700 blastocysts) was calculated considering a 10% difference in live birth rate (LBR) between LAH-group and no LAH group. The primary outcome was live birth rate and the secondary outcomes were implantation, biochemical pregnancy, clinical pregnancy, miscarriage, multiple pregnancies, obstetrical and neonatal complications rates. An interim analysis based on CPR was performed including cycles between September 2018 and October 2019.
Participants/materials, setting, methods: Women ≤ 40 years and with ≤ 2 previous oocyte retrievals undergoing IVF cycles and scheduled for elective single embryo transfer (eSET) with vitrified/warmed blastocysts were considered for enrollment in each of the two units. Warmed blastocysts were randomized immediately after warming to the study group (LAH) or to the control group

(without LAH). The allocation sequence was stratified according to woman's age and blinded to the physicians and participants.
Main results and the role of chance: Since the beginning of the study, 291 women were recruited (n=145 in LAH-group and n= 146 in the control group). At the time of transfer, hatching and hatched blastocyst rates were significantly higher in the LAH group compared to the control group (respectively 55% vs 16%, p<0.001). The clinical pregnancy rate in the whole cohort was similar in LAH and control group, being 45% and 43%, respectively (p=0.81). The clinical pregnancy rate in the age group <38 years was 45% in the LAH group compared to 39% in the control group (p=0.37). In addition, in the age group ≥ 38 years, the clinical pregnancy rates were 44% in the LAH group and 56% in the control group, respectively (p=0.30).
Limitations, reasons for caution: Being an interim analysis, the sample size is not powered enough to detect differences <10% between groups. At this step, it is not possible to evaluate LBR which is the main outcome of the study.
Wider implications of the findings: This study could clarify the real potential of AH on vitrified-warmed blastocysts in terms of live birth leading to evidence-based changes in current clinical practice.
Trial registration number: NCT03623659

O-004 The euploid blastocysts produced after either follicular-phase or luteal-phase-stimulation show similar live-birth rates: a prospective multicenter study including 293 vitrified-warmed single-embryo-transfers

A. Vaiarelli[1], D. Cimadomo[1], S. Colamaria[1], E. Trabucco[2], A. Sansone[2], E. Alviggi[2], A. Della Ragione[2], F. Golia[2], F. Fiorini[3], N. Barnocchi[3], L. Papini[3], A. Conforti4, C. Alviggi[4], L. Rienzi[1], F.M. Ubaldi[1]

[1]Clinica Valle Giulia, Genera centers for reproductive medicine, roma, Italy ; [2]Clinica Ruesch, Genera centers for reproductive medicine, Naples, Italy ; [3]Genera Umbria, Genera centers for reproductive medicine, Umbertide, Italy ; [4]University of Naples- Federico II, Department of Neuroscience- Reproductive Science and Odontostomatology, Naples, Italy

Study question: Is there any difference in the reproductive competence of euploid blastocysts produced after follicular-phase-stimulation (FPS) or luteal-phase-stimulation (LPS) during vitrified-warmed single-embryo-transfers?
Summary answer: LPS-derived vitrified-warmed euploid blastocysts showed similar live-birth-rate (LBR), obstetrical and perinatal outcomes as FPS-derived ones.
What is known already: Multiple follicular waves arise during a single ovarian cycle in humans, thereby highlighting a novel folliculogenesis pattern overtaking the classic theory. Several studies conducted by numerous groups worldwide consistently showed a similar developmental competence between FPS- and LPS-derived cohorts of oocytes in terms of fertilization, blastulation, and euploidy rates. These observations supported the further implementation of unconventional protocols for ovarian stimulation in poor-prognosis patients, like patients fulfilling the Bologna criteria, advanced-maternal-age women, oncologic patients: random-start,LPS-only and FPS+LPS in the same ovarian cycle (DuoStim). Nevertheless, it still needs to be outlined whether LPS-derived blastocysts show similar reproductive competence as FPS-derived ones.
Study design, size, duration: Multicenter study conducted between October2015–March2019 including all vitrified-warmed euploid single blastocyst transfers after DuoStim with preimplantation-genetic-testing (PGT). Only first transfers of good quality blastocysts (≥BB according to Gardner and Schoolcraft's classification) were included. In the presence of euploid blastocysts produced after both FPS and LPS (n=100/293,34%), the embryo to transfer was chosen in a blinded fashion (random.org). The primary outcome was the LBR per transfer. Biochemical-Pregnancy-Loss (BPL), Miscarriage-Rate (MR) and obstetrical/perinatal outcomes were also monitored.
Participants/materials, setting, methods: DuoStim was proposed to poor-prognosis patients respecting two of these criteria: AMH≤1.5ng/ml, antral-follicle-count≤6, ≤5 oocytes from previous cycle(s), advanced-maternal-age. Ovarian stimulations were performed with recombinant-gonadotrophins in an antagonist protocol. LPS was started five days after the first retrieval. Embryos were cultured to blastocyst, underwent trophectoderm biopsy and vitrification. Only full-chromosome meiotic aneuploidies were reported. Overall, 293 first transfers were included (139 FPS- and 154 LPS-derived euploid blastocysts). All transfers were performed in an artificial cycle.
Main results and the role of chance: To achieve 80% power (α=0.05) to rule-out a 15%-difference in LBR between FPS- and LPS-derived euploid blastocysts ~300 first SETs were required. The blastocysts in the two groups were equally distributed according to morphology and day of development. The study arms were similar for age, sperm factor, kind and cause of infertility and indication to PGT. The positive-pregnancy-tests-rates were 59.7% (n=83/139) and 63.6% (n=98/154) from FPS- and LPS-derived euploid blastocysts, respectively. The BPL rates were 10.8% (n=9/83) and 9.2% (n=9/98). The MRs were 10.8% (n=8/74) and 11.2% (n=10/89). The LBRs were 47.5% (n=66/139) and 51.3% (n=79/154;p=0.6). Among patients with euploid blastocysts produced after both FPS and LPS, the LBRs were also similar (51.9% [n=28/54] and 54.3% [n=25/46]; p=0.8). Four out of 66 (6.0%) FPS-derived pregnancies showed gestational issues (one gestational diabetes, one icterus, one polyhydramnios, one embryo twinning). Five out of 79 (6.3%) LPS-derived pregnancies showed gestational diabetes. Gestational age (38.1±1.3weeks versus 38.2±1.7weeks;p=0.6), birthweight (3309±438g versus 3232±471g;p=0.4) and length (47.9±10.2cm versus 47.6±11.4cm;p=0.9) were similar. Four out of 67 (6.0%) FPS-derived new-borns showed perinatal issues (one neonatal respiratory distress involving 7 days in the neonatal-intensive-care-unit, one diaphragmatic hernia, one single kidney, one hydrocephalous).No neonatal issue has been reported among LPS-derived new-borns.
Limitations, reasons for caution: LPS-derived embryos were produced only in a DuoStim approach. The reproductive competence of poor-quality blastocysts was not assessed. The study was powered to investigate the primary outcome, hence the analysis of secondary outcomes should be considered observational.
Wider implications of the findings: The adoption of unconventional ovarian stimulation protocols for the treatment of peculiar populations of poor-prognosis infertile and oncologic patients is increasing worldwide. This study further reinforces the hypothesis that FPS- and LPS-derived embryos have comparable competence. Therefore, if required, LPS might be adopted.
Trial registration number: none

O-005 Cleavage stage embryo transfer impairs cumulative live birth rates and time to livebirth as compared to blastocyst transfer in oocyte recipients. A randomized controlled trial

E. Clua Obradó[1], I. Rodríguez[1], G. Arroyo[1], F. Martínez[1], L. Latre[1], B. Coroleu[1], N.P. Polyzos[1]

[1]USP Instituto Universitario Dexeus, Medicina de la Reproducción, Barcelona, Spain

Study question: Does embryo transfer day (D5 *vs* D3) affect cumulative pregnancy (CPR), cumulative live birth rates (CLBR) and time to livebirth (TTL) in oocyte donation programs?
Summary answer: Embryo transfer at cleavage stage(D3) results in ~20% relative reduction in CPR and 15% in CLBR, while increases the TTL as compared with blastocyst(D5) transfer.
What is known already: Blastocyst embryo transfer has been traditionally associated with very good pregnancy outcomes due to embryo self-selection after the embryonic genome activation on day 3.

In IVF/ICSI cycles among infertile women using their own oocytes, evidence from RCTs suggest that blastocyst transfer results in significantly higher live birth rates; still no difference has been identified between D3 and D5 in terms of cumulative live birth rates. However, it is unclear whether D3 or D5 embryo transfer may result in different CPR and CLBR in women included in an oocyte donation program.
Study design, size, duration: In a single-center randomized trial conducted between March 2017-August 2018, 134 oocyte recipients were randomized to cleavage stage (D3-group) or to blastocyst stage (D5-group) embryo transfer.
Participants/materials, setting, methods: Eligible women were recipients between 18-50 years, in their 1st/2nd synchronous cycle (excluding: PGT-A, implantation failure). At first consultation and prior to oocyte donation cycle, recipients were randomly allocated to D3 or D5 transfer.

Co-primary outcomes were, CPR (first pregnancy) and CLBR (first live birth) per patient, within 12 months from the first embryo transfer, considering fresh and subsequent frozen embryo transferred. In addition, we analyzed TTL (time from 1st embryo transfer until 1st livebirth).

Main results and the role of chance: Whereas 250 recipients were planned to be enrolled, the study was prematurely terminated, with an unplanned interim analysis, when 134 women completed treatment, for ethical reasons, due to the clinically and statistically significant inferior outcomes in D3 arm.

Although the number of supernumerary frozen embryos was significantly higher in D3-group (5.3±2.7) than in D5-group (3.6±2.3), pregnancy outcomes were substantially inferior in patients allocated to D3-group.

CPR were significantly lower in the D3 vs D5 group (67.2% vs. 85.9%) with a proportion difference of -18.7% 95%CI [-32.8% to -4.3%]. Similarly, CLBR were lower in the D3 vs D5 group (55.2% vs. 70.3%) with a proportion difference of -15.1% 95%CI [-30.9% to 1.6%], although results did not reach statistical significance for this outcome.

Finally, TTB was significantly longer in D3 group as compared with D5 log rank test p=0.027. In order to reach a 50% cumulative live birth rate, D3 group required 6 months more than D5 group and always D5 group maintained a higher live birth rate.

Limitations, reasons for caution: This randomized trial was prematurely terminated, with an unplanned interim analysis, due to the clinically and statistically significant inferior results for the D3 group following evaluation by the institutional review board.

Wider implications of the findings: Transfer of embryos in cleavage stage (D3) should be discouraged in oocyte recipients with good prognosis since it significantly impairs cumulative pregnancy rates, appears to reduce cumulative live birth rates, while significantly increases time to live birth and costs due to higher number of cryopreserved embryos.

Trial registration number: NCT03088735

O-006 Blastocyst versus cleavage stage transfers: who benefits?

E.K. Dirican, Ph.D[1], Ş. Olgan[1], M. Çağlar[1], M. Sakıncı[1], O. Ertürk, Kadir[1], Z. Kasabalı[1], U. Çakmaklı[1]

[1]Akdeniz University- Faculty of Medicine, Obstetrics & Gynecology- Reproductive Endocrinology and Infertility, Antalya, Turkey

Study question: What is the outcome of cleavage and blastocyst transfers with regards to transfer cancellation, embryo selection and embryo-endometrium synchronization?

Summary answer: Transfer cancellations due to extended culture did not affect the pregnancy outcomes and patients with 6 or more zygotes benefited from embryo selection.

What is known already: Despite improvements in embryo culture, there is still a concern whether the *in vitro* extended culture supports embryo development to the same extent as *in vivo* or not. In addition, transfer cancellations often result in an unpleasant patient counseling. As a precaution for transfer cancellation, some clinics offer extended culture only when abundant embryos are available. However, the relative contributions of transfer cancellation against embryo selection during extended culture to the cycle outcome have not been fully documented. On the other hand, the effects of the proposed asynchrony between the cleavage stage embryo and the endometrium are also contradictory.

Study design, size, duration: This was a single center retrospective comparative cohort study in a university based medical assisted reproduction (MAR) center. 1710 entries from January 2018 to December 2019 were evaluated. In this cohort, embryos were not transferred on weekends. Regardless of patient and cycle characteristics, Monday and Tuesday egg retrievals were called back for transfer on day 3 (d3) (n=584), Wednesday and Thursday retrievals were on day 5 (d5) (n=532). Friday retrievals were excluded.

Participants/materials, setting, methods: Fresh cycles were included and primary outcomes were clinical pregnancy and implantation rate. Cycles were stratified on the basis of zygote numbers and analyzed using the intention to treat principle. Outcome of embryos with a known implantation status were used to demonstrate the effects of transferring earlier or later on embryo development. Statistical analyses were performed with T or chi-squared tests. Logistic regression analysis was carried out to calculate the effects of confounders.

Main results and the role of chance: For the patients having 6 or more zygotes, clinical pregnancy rates were significantly higher in the d5 group compared with d3 (48.3% (72/149) vs. 25.7% (29/113), p<0.0001). A logistic regression was performed to account for most relevant confounders and transfer day was the only significant factor (OR (95% CI) = 3.12 (1.8 – 5.43), p<0.0001). The clinical pregnancy outcome of d5 versus d3 groups for 1 (11.9% (7/59) vs. 13.7% (19/139), p=0.73), 2 (18.4% (16/87) vs. 22.9% (30/131), p=0.42), 3 (34.8% (31/89) vs. 31.8% (28/88), p=0.67), 4 (26.8% (22/82) vs. 39.1% (27/69), p=0.11), 5 (36.4% (24/66) vs. 36.4% (16/44), p=1) and ≤5 zygotes (26.1% (100/383) vs. 25.5% (120/471), p=0.83) were all comparable. Patients having ≤5 zygotes were also analyzed by logistic regression and transfer day did not matter at all. Outcome of embryos with a known implantation status were compared between d5 and d3 groups and implantation rates were comparable (9.1% (13/143) vs. 12.95% (32/247) respectively, p=0.12). Although implantation rate per number of transferred embryos of d5 group was significantly higher (31.28% (183/585) vs. 20.52% (157/765), p<0.0001), the number of transferable embryos (embryos transferred plus cryopreserved) were significantly higher in d3 group (29.14% (681/2337) vs. 46% (961/2088), p<0.0001).

Limitations, reasons for caution: The retrospective nature of this study may not eliminate potential bias. On the contrary, the strength of our study is that patients were allocated to the d5 and d3 groups regardless of patient and cycle characteristics. More research is needed to prove our findings.

Wider implications of the findings: Developmental potential of embryos was neither compromised nor supported by transferring earlier or later. Embryo-endometrium synchronization and *in vitro* versus *in vivo* embryo development does not appear to make any significant contribution to treatment outcome. Embryo selection in extended culture makes sense in patients with a larger zygote cohort.

Trial registration number: not applicalble

O-007 High grade trophectoderm is associated with monozygotic twinning in frozen-thawed single blastocyst embryo transfer

Q. Zheng[1], S. Xu[1], M. Mo[1], F. Xiong1, X. Hu[1], Y. Zeng[1]

[1]Shenzhen Zhongshan Urology Hospital, Fertility center, Shenzhen, China

Study question: What specific risk factors make monozygotic splitting at the blastocyst embryo stage?

Summary answer: High grade trophectoderm (TE), but not inner cell mass (ICM) or blastocyst expansion, is associated with monozygotic splitting after frozen-thawed single blastocyst ET.

What is known already: Elective single blastocyst embryo transfer (eSET) contributes to high live-birth rate per embryo transfer cycle and low multi-pregnancy rate, and is well perceived in clinical practice. However, blastocyst transfer is considered as the major risk factor of monozygotic splitting.

Study design, size, duration: This was a retrospective observational study including a cohort of 2863 single blastocyst-transferred pregnancies between January 2011 and June 2019 in a single fertility center in South China. The study was approved by the hospital's Ethics Committee.

Participants/materials, setting, methods: Monozygotic splitting pregnancy was identified as the number of foetuses exceeded the number of gestational sacs (GSs). The incidence of splitting regarding the oocyte age, paternal age, ovarian stimulation protocol, insemination method, type of frozen cycle, Gardner grading of expansion, ICM and TE were calculated. The serum E2, P level and endometrium thickness on hCG/progesterone day, and serum HCG levels on day 11 after ET were compared between the splitting and non-splitting pregnancies

Main results and the role of chance: There were 51 splitting cases in total among the 2863 single blastocyst-transferred pregnancies, the incidence of splitting was 1.78%. High TE grade was associated with monozygotic splitting (*P*=0.022), *aOR* for grade A and B TE were 3.96 (95% CI: 1.20-13.06) and 4.24 (95% CI: 1.24-14.46) compared to that of grade C TE respectively. While the ICM grade and blastocyst expansion were not statistically significant. There were no statistically significant differences in oocyte age, ovarian stimulation protocol, insemination method, type of frozen cycle, serum E2, P and endometrium thickness between the splitting and non-splitting pregnancies. Serum hCG levels on 11 d after ET were significantly higher in the splitting cases than those in the non-splitting pregnancies (*P*=0.020). We hypothesized that increased secretion of HCG from the high grade TE may widen the implantation window and support the monozygotic splitting.

Limitations, reasons for caution: The primary limitation of this study was its retrospective nature and small sample size. The second limitation was that MZT pregnancy was mainly diagnosed by ultrasound evaluation but not conformed by the DNA profiling for the zygosity.

Wider implications of the findings: Clinicians should consider whether to counsel couples about the slightly increased risk of monozygotic splitting

associated with high grade TE. The transfer of an embryo with an optimal TE reduces monozygotic splitting.
Trial registration number: SZSM201502035; 2018YFC1003904

O-008 Day7 blastocyst: is it worth it? A systematic review and meta-analysis

G.C. Cermisoni[1], L. Corti[1], A. Alteri[1], P. Vigano'[2], E. Giacomini[3], M. Noventa[4]

[1]San Raffaele Scientific Institute - Milan - Italy, U.O. Obstetrics and Gynecology, Milan, Italy ;
[2]San Raffaele Scientific Institute - Milan - Italy, Reproductive Sciences Laboratory, Milan, Italy ;
[3]San Raffaele Scientific Institute - Milan - Italy, Reproductive Sciences Lab - Division of Genetics and Cell Biology, Milan, Italy ;
[4]University of Padua, Department of Woman and Child Health-, Padua, Italy

Study question: What are the differences in pregnancy outcomes and euploid rate between blastocysts developed on Day7 (D7) and faster blastocysts developed on Day5 (D5) or Day6 (D6)?
Summary answer: Frozen D7 blastocyst transfers are associated with lower clinical pregnancy and live birth rates than D5/6 blastocysts. Euploid rate of screened D7 blastocysts similarly lower.
What is known already: Human embryos optimally reach the blastocyst stage after five days of culture but some have a slower development. Albeit blastocysts developing on Day 7 can be viable and result in a healthy live birth, slowly developing embryos after D6 are routinely discarded. Recent studies provide evidence supporting the idea that D6 blastocysts have a decreased reproductive potential compared to D5 ones. There is however scanty evidence regarding the clinical outcomes of Day 7 blastocysts compared to blastocysts developing on D5 or D6.
Study design, size, duration: Systematic review and meta-analysis of published studies that have evaluated the effect of delayed blastulation in frozen ART cycle. The intervention group consisted of patients with blastocysts cryopreserved on D7. Searches were conducted on 28/08/2019 using the following search terms: blastocyst, Day 5, Day 6, pregnancy, implantation, live birth and embryo transfer. The primary outcome was the clinical pregnancy rate (CPR). Secondary outcomes were euploid and survival rates after thawing and live birth rate (LBR).
Participants/materials, setting, methods: The systematic review was written following the PRISMA guidelines. Sixty-three full-text articles were preselected based on title and abstract. Study selection and data extraction were performed according to Cochrane methods. The pooled results of euploid rate, CPR and LBR were compared according to the day of blastocyst development. Odds ratios (OR) with 95% confidence intervals (CI) were calculated for outcome measures. Random-effect meta-analysis was performed and a p-value less than 0.05 was considered statistically significant.
Main results and the role of chance: Data from 11 relevant articles were extracted. The meta-analysis of 7 studies showed a lower proportion of euploid embryos comparing D7 vs. D5 (OR 0.47, 95%CI 0.39-0.57; p<0.0001) with moderate heterogeneity (I_2=69%) and comparing D7 vs. D6 (OR 0.68, 95%CI 0.61-0.75, p<0.0001) with low heterogeneity (I_2=19%).

A lower proportion of CPR (evaluated in 7 studies) was evident when transfers of blastocysts frozen in D7 were compared to those of blastocysts frozen in D5 (OR 0.37, 95%CI 0.23- 0.59; p<0.0001) with moderate heterogeneity (I_2=66%). Similarly, CPR was reduced comparing D7 vs. D6 (OR 0.49, 95%CI 0.35- 0.69, p<0.0001) with moderate heterogeneity (I_2=38%). A lower proportion of LBR (evaluated in 11 studies) was found comparing transfers of blastocysts frozen in D7 to those of blastocysts frozen in D5 (OR 0.21, 95%CI 0.16-0.27; p<0.0001) with low heterogeneity (I_2=0%) and comparing D7 vs. D6 (OR 0.34, 95%CI 0.26-0.45, p<0.0001) with low heterogeneity (I_2=0%). These findings were confirmed in a subgroup of PGT-A screened embryos. A lower survival rate (evaluated in 5 studies) was demonstrated for blastocysts frozen in D7 vs. D5 (OR 0.31, 95%CI 0.11-0.82; p=0.02) with moderate heterogeneity (I_2=72%), while the comparison of D7 vs. D6 was not statistically different.
Limitations, reasons for caution: The validity of meta-analysis results depends mainly on the number and the quality and of the included studies: this meta-analysis included retrospective studies only. The low number of D7 embryo transfers and the heterogeneity of laboratory strategies are limitations of this paper.
Wider implications of the findings: The results of this original meta-analysis confirm that delayed blastulation is associated with a poorer prognosis in terms of euploid rate and pregnancy outcomes following frozen transfers. On the other hand, the results presented do not support the discharge of embryos deriving from a delayed blastulation.
Trial registration number: PROSPERO Registration Number CRD42017067270

SELECTED ORAL COMMUNICATIONS

SESSION 03: STRATEGIES TO IMPROVE THE OUTCOMES OF OVARIAN STIMULATION I

06 July 2020 Parallel 2 10:00 - 11:30

O-009 Progestins as an alternative to Gonadotropin-Releasing hormone analogues: A retrospective study comparing in vitro fertilization outcomes during follicular and luteal phase stimulation.

L. Yamakami[1], M. Nakano[1], F. Ramos[1], L. Matsumoto[1], R. Tomioka[1], O. Duarte Filho[1], V. Heinrich-Oliveira[1]

[1]VidaBemVinda, Medical department, Sao Paulo, Brazil

Study question: May progestins instead Gonadotropin-Releasing Hormone analogues (GnRH-analogues), for suppressing premature Luteinizing Hormone (LH) surge, affects embryo viability and *in vitro* fertilization (IVF) outcomes?
Summary answer: Ovarian stimulation using Progestins does not affect chromosomal rearrangements and pregnancy outcomes; however, it increases the fertilization rate.
What is known already: The use of progestin during ovarian stimulation is effective in preventing LH surge. Development of this protocol is supported by the freezing all strategy and by multiple follicular recruitment waves, respectively, preventing detrimental effects of progesterone on embryo implantation odds, and enabling greater flexibility to physicians and patients. Nonetheless, although it has been demonstrated that progestin does not influence neither the number or the quality of the oocytes, greater depth about the embryo viability and pregnancy achievement is required.
Study design, size, duration: This retrospective study included data from 140 "freeze-all" cycles conducted between August/2018 and December/2019 and split according to pituitary suppression regimen: Gonadotropin-releasing hormone antagonists at follicular phase (GnRH-antagonists, n=53), Progestin at follicular phase (FP, n=38), and Progestin at luteal phase (LP, n=49). We consider follicular phase as day cycle 2 or 3 and luteal phase was defined based on features of transvaginal ultrasound performed after presumed ovulation.
Participants/materials, setting, methods: Women received gonadotropins for follicular recruitment and their respective method for pituitary suppression. Laboratorial procedures were conducted according to standard practice. When indicated, a blastocyst biopsy was performed for 24-chromosome analysis by Next-Generation Sequencing (NGS). The effects of stimulation protocols on IVF outcomes and embryo impairments in terms of chromosomal rearrangements were evaluated using adjusted general linear models. SPSS Statistics 20 was used for data analysis and an α of 5% was adopted.
Main results and the role of chance: There were no differences between groups regarding to female age, body mass index, basal follicle stimulation hormone (FSH), anti-müllerian hormone (AMH) and antral follicle count. Total gonadotropin doses were different between GnRH-antagonist and progestin groups (GnRH-antagonist: 2199±719 vs FP: 2512± 543; p=0.04 and LP: 2695±438; p<0.01). Twenty-nine cycles (54%) from GnRH-antagonist group, twenty-seven (71%) from FP group and twenty-six (53%) from LP group had their embryos undergoing to NGS. The euploidy rate was similar among the tree protocols, as well as the biochemical and clinical pregnancy rates. However, fertilization rate was significantly higher in the FP (86.0%) and LP (90.5%) groups when compared to GnRH-antagonists (86.0% vs 90.5% vs 72.9%, respectively, p<0.01) with Pearson' correlation (r=0.43). There was no difference among the three groups regarding endometrial priming protocol and mean endometrial thickness for frozen embryo transfers.

Limitations, reasons for caution: The present study has limitations inherent to this retrospective design, as a wide range of patient ages, as so different drugs for ovarian stimulation and triggering. Moreover, it was a single center study with a relatively small cohort.
Wider implications of the findings: Progestin use for pituitary suppression, either in follicular and luteal phases, does not affect euploid blastocyst or clinical pregnancy rates, which go against the belief that high levels of progesterone during ovarian stimulation impair oocyte and embryo quality. In addition, we highlighted that progestins provides better oocyte competence for fertilization.
Trial registration number: not applicable

O-010 Can serve Medroxiprogesterone acetate (MPA) as pituitary suppressor instead of GnRH antagonist during ovarian stimulation (OS) in oocyte donation (OD) cycles trigger with GnRH agonist?

J. Giles[1], P. Alama[1], P. Gamiz[2], C. Vidal[1], P. Badia[1], E. Bosch[1]
[1]IVIRMA Valencia, Human Reproduction, Valencia, Spain ;
[2]IVIRMA Valencia, IVF Laboratory, Valencia, Spain

Study question: Is ovarian response of oocytes donors when pituitary is suppressed with MPA comparable to the conventional treatment with GnRH antagonist cycles?.
Summary answer: MPA serve as pituitary suppressor during OS in OD since it does not present lower number of MII or worse reproductive outcome compared to antagonist.
What is known already: Administering progestins orally in the follicular phase since the beginning of OS is an efficient alternative to prevent LH from peaking, related to its effect on LH pulse frequency and amplitude**,** with similar results to conventional protocols.

Progestins have been successfully used in normo-ovulating patients, Polycistic Ovarian Syndrome, endometriosis and low-responders.

A randomized controlled trial (RCT) in OD reported no differences with GnRH antagonist in OS parameters, mature oocytes and top quality embryos. However, pregnancy outcomes were lower.

In contrast, no differences were observed between the two groups in terms of reproductive outcomes in other recently published retrospective studies.
Study design, size, duration: University-affiliated infertility clinic. Prospective RCT study, from October 2017 to June 2019, to evaluate ovarian response in terms of number of oocytes. We randomized 318 donors in two groups in a 1:1 ratio. A difference of ± 3 oocytes respect a mean of 21 in the reference group was considered as an equal response (NCT03300960).

Cycle outcome of the recipients were later analysed retrospectively. Oocytes obtained were assigned to 364 recipients (1910-VLC-091-JG).
Participants/materials, setting, methods: In MPA group 161 participants received intervention (10 mg daily administered orally during OS) and 156 were treatad with antagonist (started once the leading follicle reached 13 mm). Transvaginal ultrasound and serum estradiol (E2), LH, and progesterone (P) were performed during monitoring controls.

Other parameters that were analyzed: endocrine profile (in serum and follicular fluid), number of MII, pregnancy outcomes. For the latest, a questionnaire was offered to all participants after the oocyte retrieval.
Main results and the role of chance: No significant differences were observed in donor demographic characteristics. The number of oocytes retrieved were 21.41±11.71in the MPA group vs. 21.26±9.27 in the antagonist group (P=0.949) (Mean difference 0.14 [95%CI= -2.233, 2.517]).

The total dose of rFSH, length of OS and endocrine profile in follicular fluid in the oocyte pick-up procedure (FSH, estradiol, LH, progesterone) were comparable between groups. LH values on the day of trigger were significantly lower in study group (1.8±2.0 vs 0.9 ±1.1, p=<0.001), while no early luteinization was observed in either group.

No differences between groups were observed for implantation rate (78% vs. 73.9% p=0.441), clinical pregnancy rate (78.3% vs. 73.3, p=0.383), ongoing pregnancy rate (70.9 vs. 67%, p=0.592) and early pregnancy loss (9.7% vs. 8.0%, p=0.669). Live birth rate would be presented at the congress since there are still gestations in progress

There is a significant difference in favor of the MPA group in questions related to ease of administration and number of injections. In donors with previous cycle with antagonists level of satisfaction has been very high / high with respect to the previous cycle in 92.74%.
Limitations, reasons for caution: This is a non-inferiority study with number of retrieved oocytes as the primary outcome. The limitations of this RCT include that treatment could not be blinded, because of the different administration route of the medication in study. Another limitation to take into account is that oocyte recipients were not randomized.
Wider implications of the findings: We observed comparable oocyte retrieval, endocrine profile, viable embryo numbers and similar pregnancy outcomes in the two groups. Therefore, MPA is useful for OS in OD and provides a more friendly approach.
Trial registration number: NCT03300960

O-011 Optimising follicular development, down regulation, triggering and luteal phase support during in vitro fertilisation (IVF): a Delphi consensus

C. Alviggi[1], C. Venetis[2], H. Fatemi[3], T. D'Hooghe[4], E. Gravotta[5], M. Horton[6], M. Grynberg[7], R. Fischer[8], S. Longobardi[9], S. Esteves[10], S.K. Sunkara[11], Y. Li[12], Y. Koloda[13], R. Orvieto[14]
[1]Universita Federico II - University of Naples, Department of Neuroscience Reproductive Science and Odontostomatology, Napoli, Italy ;
[2]UNSW Australia, Centre for Big Data Research in Health, Sydney, Australia ;
[3]IVI Middle-East Fertility Clinic, Abu Dhabi & Dubai and Muscat Royal Marina Village, Abu Dhabi, United Arab Emirates ;
[4]Merck KGaA, Global Medical Affairs Fertility, Darmstadt, Germany ;
[5]Merck Serono S.p.A, Medical Affairs Fertility- Endocrinology and General Medicine, Rome, Italy ;
[6]Pregna Medicina Reproductiva, Head- Assisted Reproduction Lab and Quality Control, Buenos Aires, Argentina ;
[7]Hôpital Antoine Béclère, Department of Reproductive Medicine & Fertility Preservation, Clamart, France ;
[8]MVZ Fertility Center Hamburg GmbH, Medical Director of the Fertility Center, Hamburg, Germany ;
[9]Merck KGaA, Global Clinical Development Fertility, Darmstadt, Germany ;
[10]ANDROFERT- Andrology and Human Reproduction Clinic, Referral Center for Male Reproduction, Campinas, Brazil ;
[11]King's College London- UK, Division of Women's Health- Faculty of Life Sciences and Medicine, Barking, United Kingdom ;
[12]Beijing Chaoyang Hospital affiliate to the Capital Medical University, Reproductive Medical Center for Human, Beijing, China ;
[13]Russian Medical Academy of Continuous Professional Education, Department of Obstetrics and Gynecology, Moscow, Russia C.I.S. ;
[14]Sheba Academic Medical Centre Hospital, Director- Infertility and IVF unit, Ramat Gan, Israel

Study question: How can outcomes of *in vitro* fertilisation (IVF) be improved using the evidence-based opinion of clinical experts?
Summary answer: Eighteen statements were developed concerning improving outcomes of IVF; 17 statements reached consensus during the first vote and one reached consensus after a second vote.
What is known already: The ESHRE 2019 Guidelines provide clinicians with valuable evidence-based recommendations to optimise ovarian stimulation and IVF. However, data from such guidelines are primarily based on randomised controlled trials (RCTs) with highly selected populations that are conducted under very controlled conditions. Furthermore, these trials are limited by the fact that only ~35% of the general patient population has been reported to meet their inclusion criteria.
Study design, size, duration: A Delphi consensus was conducted to formulate expert opinion on how IVF outcomes could be improved. Step 1: statements/supporting references were discussed/amended by 11 experts. Step 2: 35 experts voted on their level of agreement/disagreement with each statement. Consensus was reached if the proportion of participants agreeing/disagreeing with a statement was >66%. If consensus was not achieved, the statement was revised and re-voted until consensus was reached. Step 3: consensus results communicated to participating experts.
Participants/materials, setting, methods: Step 1 involved the Scientific Board, comprising the Scientific Coordinator, who developed the initial statements and supporting references (which included RCTs, meta-analyses, systematic reviews, as well as retrospective studies and review articles), and 10 additional experts. The Scientific Board discussed and refined the final statements and references. Step 2

involved 35 experts who rated their level of agreement or disagreement with each statement and were asked to provide reasons for their rating.
Main results and the role of chance: Consensus was achieved for 18 statements, the most relevant of which are summarised below:

- Follicular development/gonadotropins (n=9 statements):
- Oocyte number and live birth rate (LBR) are strongly correlated; there is a positive linear correlation with cumulative LBR
- Exogenous FSH alone is sufficient for follicular development in normogonadotropic patients aged <35 years
- Different FSH preparations have identical polypeptide chains but different glycosylation patterns, affecting the biospecific activity of r-hFSH
- rLH supplementation demonstrates improved pregnancy rates and cost efficacy versus hMG in patients with severe FSH/LH deficiency
- Pituitary suppression (n=2):
- GnRH antagonists are associated with lower rates of any grade OHSS and cycle cancellation versus agonists
- Final oocyte maturation triggering (n=4):
- hCG represents the gold standard in fresh cycles
- GnRH agonist trigger, in GnRH antagonist protocol, is recommended for final oocyte maturation in women at risk of OHSS
- Current evidence supports significantly higher pregnancy rates with hCG + GnRH agonist versus hCG alone, but further evidence is needed
- The efficacy of hCG triggering for frozen transfers in modified natural cycles is controversial compared with LH peak monitoring
- Luteal-phase support (n=3):
- Vaginal progesterone therapy represents the gold standard for luteal-phase support

Limitations, reasons for caution: The statements only represent the collective opinion of the experts included. Furthermore, not all statements reached 100% agreement, with some statements reaching consensus even though some participants disagreed with them.
Wider implications of the findings: This Delphi consensus provides a real-world clinical perspective from a diverse international group of experts. Additional guidance from clinicians on IVF strategies could complement guidelines and policies, and may help to further improve treatment outcomes.
Trial registration number: not applicable

O-012 Predicted high-responder women diagnosed with oligoovulation may benefit from stimulation with highly purified human menopausal gonadotrophin (HP-hMG)

P. Heiser[1], E. Foster[1], A. Sinha[1], O. Elci[1], G. Daftary[2]

[1]Ferring Pharmaceuticals- Inc., Clinical Development, Parsippany, U.S.A. ;
[2]Ferring Pharmaceuticals- Inc., Medical Affairs, Parsippany, U.S.A.

Study question: Do gonadotrophin-related differences in efficacy exist in predicted high-responder women undergoing assisted reproductive technology based upon underlying infertility diagnosis?
Summary answer: Ongoing pregnancy rates after fresh blastocyst transfer are significantly higher with HP-hMG versus recombinant follicle stimulating hormone (rFSH) in predicted high-responder women with oligoovulation.
What is known already: Women predicted to be high-responders are a heterogenous cohort with diverse infertility etiologies and unique treatment challenges. Post-hoc analyses of previous trials suggest that these patients exhibit differential treatment response based on type of gonadotrophin administered. Use of HP-hMG is associated with fewer oocytes retrieved, higher pregnancy rates, and fewer interventions for ovarian hyperstimulation syndrome (OHSS) versus rFSH (Anckaert 2012, Arce 2012, Arce 2013). These findings required further evaluation through prospective clinical trials.
Study design, size, duration: Planned analysis of a multicenter, randomised, assessor-blind, controlled non-inferiority trial in 620 women, 21-35 years, with BMI 18-30 kg/m^2 and serum anti-Mullerian hormone (AMH) ≥35.7 pmol/L undergoing intracytoplasmic sperm injection and single blastocyst transfer. Patients were randomised to a 150IU dose of rFSH (N=309; GONAL-F, Merck) or HP-hMG (N=311; MENOPUR, Ferring), receiving 150 IU daily for the first five days with 75 IU adjustments permitted thereafter in a gonadotrophin releasing hormone (GnRH) antagonist protocol.
Participants/materials, setting, methods: Oocyte maturation was triggered with human chorionic gonadotrophin except for those with OHSS risk where GnRH agonist was substituted and fresh transfer cancelled. The primary endpoint was ongoing pregnancy rate (presence of at least 1 intrauterine pregnancy with fetal heartbeat 8-9 weeks after fresh transfer). Planned sensitivity analyses in the modified intent-to-treat population (all randomized subjects who received at least 1 dose of gonadotrophin) included assessment of primary endpoint rate by infertility diagnoses.
Main results and the role of chance: The non-inferiority objective for the primary endpoint of ongoing pregnancy was met. HP-hMG was associated with numerically higher ongoing pregnancy rates vs rFSH (35.5% vs 30.7%, P>0.05). The average number of oocytes per patient (±SD) in the rFSH arm (22.2±11.54) was higher than in the hMG arm (15.1±10.12), a difference in ovarian response that was accompanied by statistically significant increases in rates of OHSS (21.4% vs 9.7%; p<0.05). There were no significant differences in ongoing pregnancy rate between treatment groups in those diagnosed with endometriosis, male factor, tubal infertility, idiopathic, or other. However, among those diagnosed with oligoovulation, HP-hMG treatment (N=50) was associated with a 19.2% higher ongoing pregnancy rate (95% confidence interval 1.2%-37.3%) than rFSH (N=56). Relative to the rest of the trial population, those with oligoovulation had higher mean AMH (52.10 vs. 60.95 pmol/L, p<0.001), luteinizing hormone (6.45 vs 7.55 U/L, p=0.007), and testosterone (1.00 vs. 1.14 nmol/L, p=0.006) although FSH and BMI were similar. Comparisons between populations with and without oligoovulation were made using either t-tests (continuous parameters) or Fisher's exact test (categorical parameters). HP-hMG differs from rFSH in its FSH isoforms and presence of LH activity; either or both of which could account for differences observed.
Limitations, reasons for caution: The present trial was not powered to detect differences in pregnancy outcomes based upon infertility diagnoses. Additional comparative trials are required to confirm this finding.
Wider implications of the findings: The current study highlights a possible opportunity for optimization of stimulation protocols for predicted high-responders with oligoovulation through the use of HP-hMG.
Trial registration number: NCT02554279

O-013 A drop in serum progesterone levels on day of fresh blastocyst transfer, using standard luteal phase support is associated with significantly lower ongoing pregnancy rates

E. Uyanık[1], M. Polat[2], S. Mumusoglu[1], I. Yarali Ozbek[3], G. Bozdag[1], P. Humaidan[4], H. Yarali[1,2]

[1]Hacettepe University, Obstetrics and Gynecology, Ankara, Turkey ;
[2]Anatolia IVF and Women Health Center, Obstetrics and Gynecology, Ankara, Turkey ;
[3]Anatolia IVF and Women Health Center, Histology and Embryology, Ankara, Turkey ;
[4]Aarhus University- Skive Hospital, Obstetrics and Gynecology, Skive, Denmark

Study question: Do early- and mid-luteal serum progesterone (P_4) levels impact ongoing pregnancy rates (OPRs) in fresh blastocyst transfer cycles, using a standard luteal phase support (LPS)?
Summary answer: A drop in serum P_4 level from oocyte-pick up (OPU)+3 to OPU+5, using a standard LPS, is associated with a 2.8 fold decrease in OPR.
What is known already: In fresh embryo transfer cycles, significant inter-personal variation occurs in serum P_4 levels during the luteal phase, possibly due to differences in endogenous P_4 production from corpora lutea after hCG trigger and/or differences in bioavailability of exogenously administered P_4 via different routes. Although exogenous P_4 may ameliorate this drop in serum P_4 in fresh transfer cycles, there is paucity of data exploring the possible impact on reproductive outcomes of a reduction in P_4 levels.
Study design, size, duration: Using a prospective cohort study design, a total of 101 patients were consecutively enrolled between February 2019 to November 2019. The inclusion criteria were; female age <40 yr-old, retrieval of ≥3 oocytes irrespective of ovarian reserve, number of previous failed cycles ≤2, body-mass index (BMI) < 35 kg/m^2, single or double fresh blastocyst transfer. Each patient was included only once. The primary outcome measure was OPR, as defined by pregnancy ≥12 weeks of gestational age.
Participants/materials, setting, methods: A GnRH-agonist (n=23) or GnRH-antagonist (n=78) protocol was used. rhCG was used for trigger in the majority of cycles (n=95). Vaginal progesterone gel (Crinone 8%, Merck) once

daily was used for LPS. Serum P_4 levels were measured in patients on five occasions; on the day of ovulation trigger, day of OPU, OPU+3, OPU+5 and OPU+14 days; the timing of blood sampling was standardized to be 4-5 hours after the morning administration of vaginal gel.

Main results and the role of chance: Female age, BMI, number of previous cycles, number of oocytes, number and quality of blastocysts transferred were comparable among patients with (n=48) or without (n=53) OP. Similarly, mean P4 level on the day of trigger, day of OPU, and OPU+3 was comparable between two groups. However, patients with OP had significantly higher P4 levels on OPU+5 (103.6±34.0 vs 86.9±37.5 ng/ml, p=0.021). More importantly, a drop in P4 level from OPU+3 to OPU+5 was seen in 35% of patients (negative-Δ= OPU+5 – OPU+3), and was associated with a significantly lower OPR when compared with positive-Δ counterparts (31.4% vs 56.3%;OR= 2.80 (95% CI; 1.17-6.68; p=0.02); this decrease in OPR was due to lower initial pregnancy rates rather than increased early pregnancy loss rates. For negative-Δ patients, magnitude of negative-Δ was a significant predictor of OP (AUC=0.80; 95% CI; 0.65-0.96); with an optimum threshold of -18.1 ng/ml, sensitivity and specificity were 54.2% and 90.9%, respectively. Of interest, for positive-Δ patients, magnitude of Δ was not a predictor of OP (AUC=0.51; 95% CI; 0.37-0.66). When logistic regression analysis was performed, only negative–ΔP4, but not serum P4 level on OPU+5 , was noted to be an independent predictor of ongoing pregnancy (OR= 0.34;95% CI; 0.11-0.99;p=0.047).

Limitations, reasons for caution: The physiological circadian pulsatile secretion of P_4 during the mid-luteal phase is a limitation when analyses are based on one blood sample, only.

Wider implications of the findings: Two measurements (OPU+3 and OPU+5days) of serum P_4 may delineate those patients with a drop in P4 (~ 35% of whole cohort), associated with 2.8 fold lower OPRs. Rescuing these IVF cycles with additional supplementation of P_4 or adopting a freeze-all policy should be tested in future randomized controlled trials.

Trial registration number: NCT04128436

O-014 Testosterone priming (short or long course) before IVF does not improve the number of oocytes retrieved in poor ovarian responders: a randomized controlled trial.

J. Subira[1,2], A. Algaba[1], S. Vazquez[1], R. Taroncher Dasi[1], S. Monzó Fabuel[1], V. Baydal[1], A. Ruiz Herreros[1], N. Garcia Camuñas[1], J.M. Rubio Rubio[1]

[1]University Hospital La Fe, Assisted Reproduction, Valencia, Spain ; [2]IVIRMA Castellon, Assisted Reproduction, Castellon, Spain

Study question: Does follicular preparation with testosterone increase the number of metaphase II oocytes retrieved in poor ovarian responders (POR) according to Bologna criteria?

Summary answer: The use of testosterone either in a short or long course before IVF does not increase the number of MII oocytes retrieved.

What is known already: POR is characterized by an androgen-depleted follicular environment. Follicular preparation with testosterone has been used in several studies showing an increase in oocyte recovery in some of them while in others no benefit was shown. Recently, some have advocated that the possible effect of testosterone would only be achieved when given for several weeks prior to IVF, given the duration of folliculogenesis in women. Most of the studies published have limited the use of testosterone to the previous luteal phase before starting ovarian stimulation and/or were not randomized. Thus, follicular testosterone preparation in POR remains a controversial intervention.

Study design, size, duration: Randomized controlled trial comparing three groups: long-testosterone (testosterone transdermal gel 12.5 mg/day during previous two cycles), short-testosterone (testosterone transdermal gel 12.5 mg/day during previous luteal phase) and control group (no testosterone). Single-blinded for physicians involved. Sample size powered to detect a difference of at least 2 MII: 21 patients per group (N=63). Serum androgen determination at randomization, before starting stimulation and on the day of trigger. Follow-up until 12th week of gestation.

Participants/materials, setting, methods: University hospital La Fe. POR patients according to Bologna criteria. Short-antagonist protocol, fixed dose 300 IU hMG throughout stimulation. Primary outcome: number of MII retrieved. Secondary outcomes: serum androgen levels at the start of stimulation, antral follicles at the start of stimulation, number of follicles on the day of trigger, cancellation rate, embryo quality, clinical pregnancy rate. Statistical test: Poisson regression.

Main results and the role of chance: Forty-nine patients (group long-testosterone= 17, short-testosterone= 17, control= 14) completed the study as 14 out of the 63 randomised abandoned or were excluded due to several reasons. Basal characteristics of the patients were as follows: age (36.51+/-2.99), BMI (23.21+/-3.6), AMH (4.37 pmol/L+/- 2.54) and days of stimulation (10.15 +/- 2.26). There were no differences between groups. Testosterone levels and free androgen index at the time of starting stimulation were significantly higher in groups receiving testosterone compared to controls. There were no differences between groups for androstendione, SHBG or DHEA. Mean number of oocytes retrieved was 3.8 +/- 3.17 and mean number of MII was 2.56 +/- 2.68. For the primary outcome there were no differences between groups (long-testosterone=2.12+/-2.66, short-testosterone 2.71+/-2.95, control 2.92+/-2.43, p=0.98). The rest of results are still under analysis so we can only report on the primary outcome at this moment. Shortly we will have the remainder of results regarding cycle parameters and outcomes and we will update this abstract accordingly.

Limitations, reasons for caution: The drop-out rate was higher than expected (22%, sample size calculated for 15%) which could affect the power to detect differences. We present only partial results regarding mainly the primary objective.

Wider implications of the findings: Based on these preliminary results the use of testosterone in POR, either in a short or long course does not appear to increase the number of MII retrieved and therefore should not be considered as a priming strategy.

Trial registration number: NCT03378713

SELECTED ORAL COMMUNICATIONS

SESSION 04: MALE FERTILITY RELATED PREDICTORS AND THEIR USE

06 July 2020 Parallel 3 10:00 - 11:30

O-015 Paternal contribution to embryo morphokinetics in a time-lapse incubator system

A. Setti[1,2], D. Braga[1,2], R. Provenza[3], A. Iaconelli Jr.[4], E. Borges Jr.[2,4]

[1]Fertility Medical Group, Scientific research, São Paulo, Brazil ; [2]Instituto Sapientiae- Centro de Estudos e Pesquisa em Reprodução Assistida, Scientific research, São Paulo, Brazil ; [3]Fertility Medical Group, Andrology laboratory, São Paulo, Brazil ; [4]Fertility Medical Group, Clinical department, São Paulo, Brazil

Study question: Can paternal age and semen quality influence embryo morphokinetic events in a time-lapse incubator system (TLS)?

Summary answer: Embryo morphokinetic parameters are negatively influenced by paternal age and positively influenced by the seminal quality.

What is known already: Although male-factor infertility is known to play a role in 50% of the cases of infertility, the impact of male partner characteristics on IVF is often ignored. The sperm contribution to the success of IVF shouldn't be underestimated, however, few studies have focused on the influence of male factors on IVF, with conflicting results. The TLS provides the opportunity of multiple observations of the embryo developmental changes, while optimal culture conditions are maintained. Therefore, the identification of morphokinetic events affected by paternal factors may contribute to a better understanding of morphologic mechanisms in fertilization and behavior of early human embryos.

Study design, size, duration: Kinetic data were analyzed in 139 patients and 1220 zygotes cultured until day five in a TLS between March/2019 and November/2019. Timing of specific events from the point of insemination was determined using time-lapse imaging. Abnormal cleavage patterns, such as reverse cleavage and direct uneven cleavage, and the presence of multinucleation were also recorded. Multivariate linear regression analyzes were used to evaluate the influence of paternal factors on embryo morphokinetic events.

Participants/materials, setting, methods: This study was performed in a private university–affiliated IVF center. Recorded kinetic markers were: pronuclei

appearance (tPNa), timing to pronuclei fading (tPNf), timing to two (t2), three (t3), four (t4), five (t5), six (t6), seven (t7), and eight cells (t8), and timing to blastulation (tB). Durations of the second (t3-t2) and third (t5-t3) cell cycles (cc2 and cc3, respectively) and timing to complete synchronous divisions s1 (t2-tPNf), s2 (t4-t3), and s3 (t8-t5) were also calculated.

Main results and the role of chance: The paternal age was directly correlated with longer t2 (B: 0.043, p: 0.024), t3 (B: 0.056, p: 0.044), t4 (B: 0.066, p: 0.012), t6 (B: 0.080, p: 0.042), tB (B: 0.120, p: 0.041), and the presence of multinucleation (Exp(B): 1.027, p: 0.004), while the implantation rate (B: -1.933, p<0.001) and the odds of pregnancy rate (Exp(B): 0.784, p<0.001) were negatively affected by paternal age. The progressive sperm motility was negatively correlated with t4 (B: -0.016, p: 0.037), t6 (B: -0.028, p: 0.027), t7 (B: -0.044, p: 0.003), t8 (B: -0.054, p: 0.002), s3 (B: -0.033, p: 0.019), and tB (B: -0.033, p: 0.019). In addition, the total motile sperm count was inversely correlated with t8 (B: -0.009, p: 0.021), S3 (B: -0.009, p: 0.005), and tB (B: -0.011, p:0.019).

Limitations, reasons for caution: Retrospective nature of this study and the small sample size may be a reason for caution.

Wider implications of the findings: The association between embryonic growth rate and embryo quality is well recognized. Implantation and live-birth rates are lower with slow-growing embryos. We demonstrated that increasing paternal age and poor seminal quality correlates with delayed cell cleavage and blastulation. This finding highlights the importance of paternal contribution for the IVF success.

Trial registration number: Not Applicable

O-016 Low male testosterone results in a substantial decrease of fresh live birth rates in couples with non-male factor infertility undergoing IVF

P. Drakopoulos[1], F. Di Guardo[1], C. Blockeel[1], M. De Vos[1], E. Anckaert[1], G. Verheyen[1], S. Santos-Ribeiro[2], A. Racca[1], L. Boudry[1], S. Mackens[1], H. Tournaye[1], V. Vloebergs[1]

[1]UZ Brussel, Center for Reproductive Medicine, Jette- Brussels, Belgium ; [2]IVI-RMA, Department of reproductive medicine, Lisbon, Portugal

Study question: Are male testosterone levels associated with reproductive outcomes in couples with non-male factor infertility undergoing IVF?

Summary answer: Low male serum testosterone levels in couples with non-male factor infertility are associated with a decrease in fresh live birth rates after fresh embryo transfer

What is known already: Low serum testosterone is found in approximately 15% of subfertile men. Although testosterone is essential in spermatogenesis, it is unclear whether low testosterone levels may have a negative impact on reproductive outcomes in couples with non-male factor infertility. Furthermore it is debatable whether the initial evaluation of the subfertile male should include an endocrine assessment

Study design, size, duration: This was a 7 years (2011 to 2018) retrospective, single-center cohort study conducted at a tertiary fertility clinic.

Participants/materials, setting, methods: All couples with non-male factor infertility who underwent their first IVF cycle in a GnRH antagonist protocol were included. All asymptomatic men provided morning blood samples (before 10 am) and none had been on exogenous testosterone or other relevant medication. Low total testosterone (TT) was defined as TT<264 ng/dL in line with the recently published guidelines of the endocrine society. Testosterone assay used was the same for all patients.

Main results and the role of chance: In total 1026 couples were included in the analysis. Among them, 136 (13.3 %) had low TT. Semen concentration, progressive (A+B) motility (WHO 2010) and morphology using Kruger's strict criteria were lower, but not significantly different between patients with low and normal TT [median (IQR): 52.8 x 10^6 (28-109) vs. 54.3 x 10^6 (27-93), 58% (41-68) vs. 59 (45-69) and 5% (4-8) vs. 6% (4-10), p value=0.6, 0.3 and 0.3, respectively]. However, fresh live birth rates (LBR) after fresh embryo transfer were significantly lower in patients with low TT (13.2% vs. 23.2%, p value=0.009). Multivariate regression analysis allowing adjustment for relevant confounders revealed that TT status was significantly associated with fresh LBR. The odds of fresh LB decreased by 65% in couples whose male partner had low TT (adjusted OR =0.35, 95% CI=0.15-0.79,p value=0.01). The results were also replicated when TT was considered as a continuous variable and when free calculated testosterone was used in the regression model

Limitations, reasons for caution: This is a large observational study based on retrospective data collection. Despite our robust methodological approach, the presence of biases related to retrospective design cannot be excluded

Wider implications of the findings: Our findings underscore the importance of a complete endocrine evaluation of the subfertile male. Efforts to evaluate and maybe optimize even asymptomatic low TT in male partners should be considered. Future research might need to identify a new cut-off in the definition of "normal" TT, in an infertility context

Trial registration number: N/A

O-017 Negative impact of elevated DNA fragmentation index (DFI) and human Papillomavirus (HPV) presence in sperm on the outcome of intra-uterine insemination (IUI)

C. Depuydt[1], G. Donders[2], L. Verstraete[1], J. Beert[1], G. Salembier[1], E. Bosmans[1], D. Nathalie[3], W. Ombelet[3]

[1]AML- Sonic Healthcare, Department of Hormonology and Reproductive Health- AML, Antwerp, Belgium ; [2]University Hospital Antwerpen, Department of Obstetrics and Gynecology, Antwerp, Belgium ; [3]Genk Institute for Fertility Technology- ZOL Hospitals, Zorgprogramma Reproductieve Geneeskunde B ZRGB, Genk, Belgium

Study question: To determine the impact of DFI and HPV-DNA positivity on fertility outcome (biochemical and clinical pregnancy rate) in sperm before its use in IUI.

Summary answer: DFI and HPV status in sperm from male partners are independent predictors of clinical pregnancies (CP) in women undergoing IUI.

What is known already: Recent evidence identified HPV infections as a possible cause of male and couple infertility in IUI. While the pathogenesis of HPV infection in the cervix can be divided in two pathways, (an infectious, virion-producing and a non-infectious, cell transforming, cancer-inducing pathway), in sperm, the HPV DNA always originates from infectious virons only and is limited in time. HPV virions can bind syndecan-1 present at two distinct sites along the equator of the spermatozoon's head, not only causing detrimental effects on sperm parameters but also damages the DNA of the spermatozoids, impacting on gamete interaction and causing temporal subfertility.

Study design, size, duration: Non-interventional prospective multi-center study (12-month study period) in which we measured both the DFI and HPV DNA in sperm before its use in IUI in a cohort of 161 consecutive infertile couples (209 IUI cycles).

Participants/materials, setting, methods: DFI was measured with the sperm chromatin structure assay and HPV DNA was detected with type specific quantitative PCRs (HPV 6,11,16,18,31,33,35,39,45,51,52,53,56,58,59,66 and 68) in sperm before its use in IUI. We analyzed the impact of DFI and HPV positivity on fertility outcome (biochemical and clinical pregnancy rate) and calculated the clinical cutoff value for DFI.

Main results and the role of chance: A DFI criterion value of 25.7% was calculated by ROC curve analysis. Couples with DFI >26% had significantly less CP than couples with DFI ≤26% (OR 31.8, 1.8-510.9, p = 0.017). In total, 31 sperm samples from 169 different men were HPV positive, resulting in an HPV prevalence of 14.8% per IUI cycle and a high-risk HPV prevalence of 9.6% per IUI cycle (20/209). HPV positive sperm samples had a significantly higher DFI compared to HPV negative sperm samples (29.8% vs 20.9%; p=0.011). None of the 31 inseminations in which the sperm tested positive for HPV led to pregnancy, even when DFI% was below 26%.

Limitations, reasons for caution: HPV positivity (virions) in female partners could also lead to DNA damage of spermatozoa and influence the clinical pregnancy rate per cycle even more. Therefore, to have a complete picture of the prediction model both partners should be tested for HPV and ideally each time an IUI cycle is performed.

Wider implications of the findings: Because men can easily infect women and these HPV infected women have longer duration of transient infections resulting in (longer) infertility period. Considering the low IUI success rates IVF or ICSI might be indicated in HPV-positive couples and in couples with a DFI above 26% to increase the pregnancy rate.

Trial registration number: EudraCT number: 2017-004791-56

O-018 Proteomic characterization of spermatozoa from patients with idiopathic infertility.

I. Urizar Arenaza[1], B. Navarro[2], B. Gómez-Giménez[1], A. Odriozola[1], S. Martín-González[1], I. Muñoa-Hoyos[1], M. Gianzo[1], T. Ganzabal[2], N. Subiran[1]

[1]University of the Basque Country UPV/EHU, Physiology, Leioa, Spain ; [2]Quirón Bilbao, Assisted Reproduction Unit., Bilbao, Spain

Study question: Which is the proteomic profile of spermatozoa belonging to patients with idiopathic infertility?
Summary answer: Compared to normozoospermic samples, we identified a total of 385 differentially expressed proteins that are involved in human sperm metabolism.
What is known already: Infertility has become a medical and social problem that affects over 186 million people worldwide and male factors represent approximately 45% of clinical cases. Among other factors, anatomical or genetic abnormalities, problems in spermatogenesis or environmental factors are responsible for abnormal sperm parameters which contribute to male infertility. However, approximately 20-30% of men with normal sperm parameters have impaired fertility caused by unknown deficiencies. Thereby, the molecular mechanisms underlying idiopathic infertility are poorly understood.
Study design, size, duration: We used 80 normozoospermic samples and 80 pathological samples belonging to men with idiopathic infertility. Male idiopathic infertility was considered when spermatozoa presented normal sperm parameters but there was a repeated failure in the Assisted Reproduction Techniques. Samples were obtained from the Quirón Bilbao Clinic and were isolated and capacitated by swim up.
Participants/materials, setting, methods: We adopted a Tandem Mass-Tag (TMT) 6-plex isotopic labeling strategy and generated samples for LC-MS/MS. We extracted proteins from normozoospermic samples and samples with idiopathic infertility and after proteomic procedures we run the generated peptides in a Q Exactive mass spectrometer. Protein searches were performed using the MaxQuant Software. Perseus software (v.1.6.0.7) was employed for the calculation of the statistical significance and fold changes between samples with idiopathic infertility and normozoospermic samples ($p<0.05$).
Main results and the role of chance: In the present study we identified a total of 1722 human sperm proteins. Differential proteomic studies showed an increase in the relative abundances of 176 proteins and a decrease in the relative abundances of 209 proteins. According to the proteins that are upregulated, there are involved in metabolical processes, being the most enriched ones the acetyl-CoA biosynthetic process, ADP transport and 2-oxoglutarate metabolic process. Regarding proteins that are downregulated, there are involved in ATP metabolic processes, protein targeting and nucleotide processes. According to our results, human sperm metabolism may be key in the finding of targets that may cause idiopathic infertility. This finding suggests that altered metabolic parameters may lead human sperm to idiopathic infertility.
Limitations, reasons for caution: We need further studies to identify and validate specific protein targets that may alter specific metabolic processes and consequently cause idiopathic infertility.
Wider implications of the findings: The study of human sperm metabolism may be essential to understand the causes of many idiopathic infertility cases. This supports the idea that proteins involved in metabolic processes could be used as therapeutic targets in the treatment of idiopathic infertility.
Trial registration number: CEISH/61/2011.

O-019 extremely low serum AMH level correlates with a high sperm retrieval rate of micro-TESE in men with idiopathic azoospermia

J. Zhang[1], X. Liang[1]

[1]the Sixth Affiliated Hospital- Sun Yat-sen University, reproductive center, Guangzhou- Guangdong, China

Study question: To investigate the value of serum anti-Müllerian hormone (AMH) level in predicting the sperm retrieval rate (SRR) of micro-TESE for patients with idiopathic azoospermia.
Summary answer: Idiopathic azoospermia cases with low AMH level (<1.0ng/ml) would have more opportunity to present heterogeneous seminiferous tubules when micro-TESE was performed and had higher SRR.
What is known already: For infertile patients with non-obstructive azoospermia (NOA), micro-TESE is considered to have higher SRR than traditional methods. However, Serum inhibin B, follicle-stimulating hormone (FSH) and various clinical parameters are not reliable predictors for the presence of focal spermatogenesis and SRR. SRR of micro-TESE between NOA patients with different etiology varies widely. Idiopathic azoospermia would have much lower SRR than orchitis, cryptorchidism, AZFc deletion, Klinefelter syndrome and other cases when micro-TESE is performed. Therefore, it is still controversial that whether micro-TESE have an advantage in patients with idiopathic azoospermia.
Study design, size, duration: This is a retrospective study. From Sept. 2014 to Jan. 2020, 163 idiopathic azoospermia patients treated with micro-TESE by single surgeon were divided into three groups according to their serum AMH level: group A <1.0ng/ml (n=41), group B 1.0-5.0ng/ml (n=68), group C >5.0ng/ml (n=54). Patient's age, testicular volume, serum FSH and AMH level, SRR and heterogeneous seminiferous tubules presenting rate were evaluated.
Participants/materials, setting, methods: Micro-TESE was performed at x10 to x20 magnification. An attempt was made to identify seminiferous tubules that were larger and more opaque than others. The procedure was terminated when sperm were retrieved. If all tubules were seen to have an identical appearance, at least three samples (upper, middle, and lower) were extracted. Venous blood samples were drawn from each patient (7–10 AM) after an overnight fast. FSH and AMH were measured by electrochemiluminescence immunoassay.
Main results and the role of chance: Testicular sperms were successfully retrieved in 55 cases (SRR=33.7%). There was no statistical difference in age, testicular volume, FSH level between the patients who succeeded and failed to obtain sperm (33.3±5.1 years, 5.4±3.0 ml, 26.7±15.7 IU/L vs. 32.5±5.7 years, 6.0±2.8 ml, 26.1±12.6 IU/L, p>0. 05 respectively). The patients who obtained sperms had lower serum AMH level than those without sperm (2.87±3.79ng/ml vs. 4.99±4.64ng/ml, p<0. 05). SRR and heterogeneous seminiferous tubules presenting rate were 56.1% (23/41), 70.7% (29/41) in group A vs. 30.9% (21/68), 27.9% (19/68) in group B vs. 20.4% (11/54), 13.0% (7/54) in group C (p<0. 05 respectively). In group A (AMH=0.36±0.29ng/ml), SRR and heterogeneous seminiferous tubules presenting rate were highest, meanwhile they were lowest in group C (AMH=9.26±4.37ng/ml).
Limitations, reasons for caution: Seminal AMH level should be included in the following study to confirm our conclusion. Randomized controlled trial comparing micro-TESE and traditional TESE would demonstrate that whether idiopathic azoospermia patients with serum AMH level higher than 1.0ng/ml could not have benefit by microsurgery.
Wider implications of the findings: Recently, in our pathologic research, NOA patients with extremely lower serum AMH level are observed to have more opportunity to present severe hyalinization in their seminiferous tubules. Tubules with severe hyalinization have less Sertoli cells and seem very thin. Therefore, tubules with spermatogenesis would be easy to identify during micro-TESE.
Trial registration number: Not Applicable

O-020 MACS Vs TESA for raised sperm DNA fragmentation index – A RCT

K.C. Mantravadi[1], D.R. Gedela[1], S. Thakur[1], P. Shetty[1]

[1]Oasis Fertility, Fertility, Hyderabad, India

Study question: In Individuals with raised Sperm DNA Fragmentation Index (DFI), sperm selection by magnetic activated cell sorting (MACS) or surgical retrieval of testicular sperms (TESA) optimize the reproductive outcomes?
Summary answer: TESA seems like a beneficial intervention to optimize sperm selection and reproductive outcomes for Individuals with raised sperm DFI.
What is known already: It is evident that raised sperm DFI negatively affects the reproductive outcomes. Management for raised sperm DFI to optimize reproductive outcomes is still elusive.
Study design, size, duration: This is an ongoing Randomised Control Trial (RCT) with prior approval from institutional Ethical Committee (IEC). This is preliminary data of the pilot study. Study duration 6months (March – October 2019). Couples undergoing IVF stimulation with raised sperm DFI were

randomised into MACS group (n=30) and TESA group (n=30) for sperm selection. Couples with history of one failed IVF cycle were offered testing for sperm DFI. Individuals with sperm DFI>30% were included in the study.
Participants/materials, setting, methods: Sperm DFI testing was done with SCSA method and randomized using software. Intra Cytoplasmic Sperm Injection (ICSI) was the method of insemination in all cases. Extended embryo culture till blastocyst stage was done and a freeze all policy was opted. Two Blastocysts that showed 100% survivals were transferred in a Frozen Embryo transfer (FET) cycle. Implantation rates (IR) and Clinical Preganacy Rates (CPR) were compared between both groups.
Main results and the role of chance: MACS group showed 38% blastocyst formation rates with a CPR of 50% and IR of 43%

TESA group showed 31% blastocyst formation rates with a CPR of 83% and IR of 72%

Though MACS group had slightly more percentage of blastocyst formation; TESA group had significantly higher CPR and IR.

Individuals with history of failed implantation and raised sperm DFI, TESA seems to be beneficial intervention to optimize reproductive outcomes.
Limitations, reasons for caution: Small sample size. TESA is a surgical intervention.
Wider implications of the findings: Testicular sperm seem to have better DNA quality than ejaculated sperm. In couples with failed IVF attempts and raised sperm DFI we can offer TESA as an active intervention to optimize reproductive outcomes.
Trial registration number: REF/2019/07/026887

SELECTED ORAL COMMUNICATIONS

SESSION 05: ENDOMETRIOSIS AND UTERINE DISORDERS. NEW CLINICAL INSIGHTS

06 July 2020 Parallel 4 10:00 - 11:30

O-021 Laparoscopic surgery for endometriosis: a Cochrane systematic review (Cochrane update).

C. Bafort[1,2], Y. Beebeejaun[3], C. Tomassetti[1,2], J. Bosteels[2,4], J. Duffy[5]

[1]UZ Leuven, OBGYN/Leuven University Fertility Center, Leuven, Belgium ;
[2]KULeuven, Dept of Development and Regeneration, Leuven, Belgium ;
[3]Guy's and St. Thomas' Hospital NHS Foundation Trust, Assisted Conception Unit, London, United Kingdom ;
[4]Imelda Hospital, Department of obstetrics and gynaecology, Bonheiden, Belgium ;
[5]University of Oxford, Nuffield Department of Primary Care Health Sciences, Oxford, United Kingdom

Study question: Does laparoscopy improve pain and/or infertility associated with endometriosis compared to any other laparoscopic or robotic intervention, holistic or medical treatment or diagnostic laparoscopy only?
Summary answer: This updated review shows that laparoscopic surgery reduces pain and improves live birth rates. When comparing different surgical techniques, no difference in outcomes was shown.
What is known already: Endometriosis is defined by the presence of endometrial glands and stroma in ectopic locations such as the pelvic peritoneum, ovaries and rectovaginal septum. Symptoms include pain and/or infertility. Treatment options for endometriosis include medical therapy (hormonal therapy and non-steroidal anti-inflammatory drugs) and surgery. Laparoscopic surgery may benefit in treating overall pain and infertility associated with mild to moderate endometriosis. Although the laparoscopic management of endometriosis is widely accepted, the ideal surgical techniques are still being debated.
Study design, size, duration: For the update of this Cochrane systematic review we conducted electronic searches of the Cochrane Gynaecology and Fertility Specialised Register of Controlled Trials, CENTRAL, MEDLINE, EMBASE, PsycINFO and CINAHL from inception to July 2019 to identify relevant randomized controlled trials (RCTs). Three independent review authors (CB, YB, CT) independently selected trials and extracted data for meta-analysis. Any disagreements were resolved by discussion or by a third review author (JD).
Participants/materials, setting, methods: Participants: Women with endometriosis confirmed with a visual diagnosis at diagnostic or operative laparoscopy. Intervention: Laparoscopic intervention. Comparison: Another laparoscopic or robotic intervention, holistic or medical intervention or diagnostic laparoscopy. Risk ratios (RR) were calculated for dichotomous data and mean differences (MD) for continuous data, with 95% confidence intervals (CI). Heterogeneity was examined via the I^2 statistic. Primary analysis was conducted on data per woman randomised. Primary outcomes: overall pain and live birth.
Main results and the role of chance: For this update, we screened 812 titles and abstracts. We identified 5 new studies (including one ongoing study). We included a total of 14 studies with 1102 women in the review. Laparoscopic surgery (ablation or excision) was associated with decreased overall pain (measured as 'pain better or improved') at 6 months (RR6.58, 95%CI3.31to13.10, three RCTs, n=171, I^2=0%, moderate-quality evidence) and 12 months postoperatively (RR10.00, (95%CI3.21to31.17, one RCT, n=69, low-quality evidence) compared to diagnostic laparoscopy only. Laparoscopic surgery was associated with an increased live birth or ongoing pregnancy rate (RR1.94, 95%CI1.20to3.16, two RCTs, n=382, I^2=0%, moderate-quality evidence).Laparoscopic surgery was associated with decreased overall pain at 12 months (measured as 'pain free at 12 months') postoperatively compared to diagnostic laparoscopy and medical treatment (RR5.63, 95%CI1.18to26.85, one RCT, n=35, low-quality evidence).On comparing laparoscopic ablation with laparoscopic excision, there was no evidence of a difference in overall pain (12 months) (MDon0to10VAS0.00, 95%CI-1.22to1.22, one RCT, n=103, low-quality evidence). Live birth was not reported in any of the included trials. We included three studies in this comparison, while one study is awaiting classification.Due to the limited available evidence it is unclear if a certain surgical technique is superior. We included 2 studies, both are awaiting classification.
Limitations, reasons for caution: The quality of some of the included studies, the heterogeneity of the disease among the different studies and limited long-term follow-up are the major limitations of this systematic review. Furthermore, there were few studies for each comparison and meta-analysis could rarely be performed.
Wider implications of the findings: Surgical (laparoscopic) treatment of endometriosis appears to beneficially influence pain and pregnancy outcomes. Further high-quality RCT are required to further differentiate between various treatment options (whether or not surgical) and their corresponding effect on the different outcomes of the core outcome set for endometriosis.
Trial registration number: NA

O-022 Long-term treatment with norethindrone acetate decreases the postoperative recurrence of deep endometriosis at long-term follow-up

S. Ferrero[1], C. Scala[2], G. Evangelisti[1], F. Barra[1]

[1]IRCCS Ospedale Policlinico San Martino - University of Genoa, Academic Unit of Obstetrics and Gynecology, Genoa, Italy ;
[2]Gaslini Hospital, Unit of Obstetrics and Gynecology, Genoa, Italy

Study question: Does postoperative treatment with norethindrone acetate (NETA) decrease the risk of endometriosis recurrence following surgical treatment of endometriosis?
Summary answer: Postoperative administration of NETA significantly decreases the risk of recurrence of both endometriosis related symptoms and ultrasonographically diagnosed endometriotic lesions at long-term follow-up.
What is known already: Progestins are widely used to treat endometriosis-related symptoms. Postoperative administration of hormonal therapies may decrease the recurrence of endometriosis-related pain and ovarian endometriomas. Limited information is available on the role of postoperative treatment with NETA in preventing recurrence of endometriosis.
Study design, size, duration: The study was performed between June 2014 and December 2019. Four hundred thirty-seven women were enrolled. The patients, after being undergone surgical excision of deep endometriosis, either received continuous postoperative treatment with NETA or did not receive any hormonal therapy, were enrolled. Patients exit the study when they interrupted the protocol started immediately after surgery.
Participants/materials, setting, methods: The choice of receiving NETA was based on the preference of the patients (including previous experiences with hormonal therapies and their adverse effect, contraindications to hormonal therapies and/or desire to conceive). Patients underwent a follow-up consultation

(including transvaginal ultrasonography) every six months. Presence and intensity of pain symptoms were assessed. Quantity of life was evaluated using the EHP30 and sexual function was evaluated using the Female Sexual Function Index (FSFI).
Main results and the role of chance: Demographic characteristics, severity of endometriosis (assessed by the rASRM classification), prevalence of endometriomas and length of follow-up were similar in patients who received postoperative NETA (n = 309) and in those who did not receive postoperative therapy (n = 128). At a median length of follow-up was 46 months (range, 12-73 months), the rate of ultrasonographic diagnosis of endometriosis recurrence was significantly lower in patients treated with NETA (16/309; 5.2%) than in those who did not receive hormonal therapy (22/128; 17.2%; $p < 0.001$). NETA significantly decreased the risk of recurrence of endometriomas ($p < 0.001$) and of deep endometriotic nodules ($p < 0.01$). NETA decreased the occurrence of *de novo* endometrioma and deep endometriotic lesions during the follow-up ($p < 0.05$). Similarly, the recurrence of endometriosis-related pain symptoms was significantly lower in patients treated with NETA (31/309; 10.0%) than in those who did not receive hormonal therapy (46/128; 35.9%; $p < 0.001$). Quality of life and sexual function were better in patients treated with NETA than in those who did not receive postoperative hormonal therapy ($p < 0.001$ and $p < 0.001$, respectively).
Limitations, reasons for caution: The study was not randomized. Only NETA was investigated in this study; it remains to be evaluated if other hormonal therapies are more efficacious than NETA in preventing postoperative endometriosis recurrence.
Wider implications of the findings: Patients undergoing surgery for endometriosis who do not desire to conceive should be advised to use postoperative hormonal therapy to decrease the risk of endometriosis recurrence.
Trial registration number: Not applicable

O-023 Relugolix combination therapy improves hemoglobin levels in anemic women with heavy menstrual bleeding due to uterine fibroids: results from the LIBERTY Phase 3 program

R. Venturella[1], E.A. Stewart[2], A. Al-Hendy[3], J. Kang[4], L. McKain[4], A. Lukes[5]

[1]University Magna Graecia, Obstetrics and Gynecology, Catanzaro, Italy ;
[2]Mayo Clinic, Department of Obstetrics & Gynecology, Rochester- Minnesota, U.S.A. ;
[3]University of Illinois/Chicago, Department of Obstetrics and Gynecology, Chicago- Illinois, U.S.A. ;
[4]Myovant Sciences Inc., Myovant Sciences Inc., Brisbane- California, U.S.A. ;
[5]Carolina Woman's Wellness Center, Obstetrics and Gynecology, Durham- North Carolina, U.S.A.

Study question: What is the impact of 24 weeks of relugolix combination therapy (Relugolix-CT) on hemoglobin in women with uterine fibroids (UF) and anemia at baseline?
Summary answer: In women with UF and anemia at baseline, Relugolix-CT, an oral gonadotropin-releasing hormone with estradiol/norethindrone, significantly improved hemoglobin concentrations at Week 24 ($p < 0.0001$).
What is known already: In the LIBERTY 1 and 2 Phase 3 studies, Relugolix-CT (once-daily relugolix 40 mg, estradiol [E2] 1.0 mg, and norethindrone acetate [NETA] 0.5 mg) significantly reduced menstrual blood loss (MBL) in women with UF-associated heavy menstrual bleeding (HMB) and was well tolerated. In the pooled analysis of Phase 3 data, the proportion of responders was 72.3% in the Relugolix-CT group (n=254) and 16.8% in the placebo group (n=257); $p < 0.0001$. Additional benefits included a clinically meaningful reduction of UF-related pain and a high rate of amenorrhea. Coadministration of E2/NETA maintained bone mineral density and minimized incidence of vasomotor symptoms.
Study design, size, duration: LIBERTY 1 and 2 were randomized, double-blind, placebo-controlled, international, Phase 3 trials to assess the efficacy and safety of Relugolix-CT for 24 weeks of treatment. Women were randomized 1:1:1 to one of three arms: Relugolix-CT for 24 weeks, Delayed Relugolix-CT (relugolix 40 mg alone for 12 weeks followed by Relugolix-CT for 12 weeks), or placebo for 24 weeks. Data were pooled from LIBERTY 1 and 2 for this analysis.
Participants/materials, setting, methods: Premenopausal women (aged 18–50 years) with an MBL volume of ≥ 80 mL/cycle (assessed using the alkaline hematin method) and ultrasound-confirmed UF were eligible. The results of an analysis of a subset of women with hemoglobin ≤ 10.5 g/dL at baseline who had a hemoglobin value at Week 24 are reported. The proportion of patients who had a 2 g/dL hemoglobin increase from baseline and an absolute percentage change from baseline hemoglobin was assessed.
Main results and the role of chance: The proportion of women with anemia was 33% (n=83) in the Relugolix-CT group and 32% (n=83) in the placebo group. The anemic women were notably different than the women in the overall study populations of LIBERTY 1 and 2. They were more likely to be Black/African American women (65.2% vs 51.2%) and from North America (85.9% vs 75.5%]). Their baseline MBL volume was greater (mean: 285.5 mL vs 228.8 mL) with larger proportion having baseline MBL volumes ≥ 225 ml (52.0% vs 34.8%) and they had a larger baseline uterine volume (mean: 488 cm^3 vs 408 cm^3).

Nonetheless, a significantly greater proportion of women had a clinically meaningful increase in hemoglobin levels of ≥ 2 g/dL in the pooled Relugolix-CT arms (55.7%) compared with the placebo arms (11.7%; $p < 0.0001$). Similarly, the mean percentage increase in hemoglobin concentration was greater in the pooled Relugolix-CT arms (23.0%) compared with the placebo arms (6.4%; $p < 0.0001$).
Limitations, reasons for caution: Patients with evidence of iron deficiency anemia, at any time during the trial, were required to receive parenteral or oral iron supplementation. However, iron therapy was not standardized. Therefore, outcomes may have been impacted by differences in methods of supplementation (oral versus parenteral) and patient adherence to therapy.
Wider implications of the findings: Overall, these significant improvements in hemoglobin concentrations from baseline with Relugolix-CT treatment relative to placebo reflect the impact that HMB had on study participants and how reductions in MBL volume translate into clinically meaningful improvements in hemoglobin levels in patients with UF.
Trial registration number: NCT03049735 and NCT03103087

O-024 Relugolix combination therapy reduced patient-reported distress from bleeding and pelvic symptoms and improved daily activities in patients with uterine fibroids in the LIBERTY program

E. Stewart[1], A. Al-Hendy[2], R. Venturella[3], A. Poindexter III[4], C. Villarroel[5], Y. Li[6], R.B. Wagman[6], E. Hunsche[6], A. Lukes[7]

[1]Mayo Clinic, Ob Gyn, Rochester MN, U.S.A. ;
[2]University of Illinois/Chicago, Department of Obstetrics and Gynecology, Chicago- Illinois, U.S.A. ;
[3]Magna Graecia University of Catanzaro, Department of Obstetrics and Gynecology, Catanzaro, Italy ;
[4]Baylor College of Medicine and St. Luke's Episcopal Hospital, Obstetrics & Gynecology, Houston- Texas, U.S.A. ;
[5]Instituto de Investigaciones Materno Infantil IDIMI- University of Chile, Reproductive Medicine Unit, Santiago, Chile ;
[6]Myovant Sciences Inc., Myovant Sciences Inc., Brisbane- California, U.S.A. ;
[7]Carolina Woman's Wellness Center, Obstetrics and Gynecology, Durham- North Carolina, U.S.A.

Study question: What is the effect of relugolix combination therapy (Relugolix-CT) on patient-reported distress from common uterine fibroids (UF) symptoms and on activities in women with UF?
Summary answer: Relugolix-CT treatment for 24 weeks significantly reduced patient-reported distress from common UF symptoms and improved physical and social activities vs placebo (nominal $p < 0.001$).
What is known already: The efficacy and safety of Relugolix-CT treatment (once-daily relugolix 40 mg, estradiol [E2] 1.0 mg, and norethindrone acetate [NETA] 0.5 mg for 24 weeks), in women with UF-associated heavy menstrual bleeding (HMB) was demonstrated in the LIBERTY 1 and 2 Phase 3 trials. Relugolix-CT achieved a statistically significant and clinically meaningful reduction in menstrual blood loss and in pain among patients with moderate to severe pain at baseline, a high rate of amenorrhea, and a clinically meaningful hemoglobin increase in patients with anemia. Furthermore, bone mineral density was maintained, and vasomotor symptoms minimized, as in the placebo group.
Study design, size, duration: 388 and 382 premenopausal women with clinically significant uterine fibroids and alkaline-hematin documented HMB, respectively, were randomized 1:1:1 to: Relugolix-CT for 24 weeks, relugolix 40 mg for 12 weeks followed by Relugolix-CT for 12 weeks, or placebo for 24 weeks. Pooled data from both studies were used for analyses of distress from common UF symptoms, measured using a new, validated patient-reported outcome tool, and of activities assessed with the previously validated UFS-QoL revised activities scale.

Participants/materials, setting, methods: The Bleeding and Pelvic Discomfort (BPD) scale consists of three UFS-QoL items assessing distress due to HMB, passing blood clots, and pelvic pressure/tightness. The Revised Activities (RA) scale contains five items relating to physical and social activities. Both scales range from 0 to 100; least squares means (95% confidence interval [CI]) for change from baseline were obtained using mixed-effects model repeated measures, and percentage of responders were analyzed for the Relugolix-CT and the placebo groups.

Main results and the role of chance: The reduction from baseline to week 24 in distress due to HMB, passing blood clots, and pelvic pressure, measured by the BPD scale, was significantly greater with Relugolix-CT (-45.0) than with placebo (-16.1) (difference -29.9; p<0.001). With Relugolix-CT, mean BPD scale scores improved from 67.5 (CI: 63.4, 71.5) at baseline to 22.7 (CI: 17.8, 27.6) at Week 24, while the placebo group changed from 71.9 (CI: 67.8, 75.9) to 55.8 (CI: 50.9, 60.6). In addition, Relugolix-CT led to an improvement in RA scale scores from 33.8 (CI: 29.3, 38.3) at baseline to 78.4 (CI: 73.2, 83.5) at Week 24, while the placebo group changed from 29.4 (CI: 24.9, 33.9) to 43.8 (CI: 38.8, 48.9); the difference between Relugolix-CT and placebo (30.7) was statistically significant (p < 0.0001). Percentages of treatment responders, defined as those with a clinically meaningful change from baseline to Week 24 of ≥ 20 points, were significantly greater with Relugolix-CT than placebo. On the BPD scale, 61.7% vs 27.6% on Relugolix-CT vs placebo were responders, with a significant difference of 34.2% (p<0.0001). Regarding the RA scale, 60.9% and 35.4% of patients on Relugolix-CT and placebo, respectively, were responders, with a statistically significant difference of 25.5% (p<0.0001).

Limitations, reasons for caution: The BPD is a new scale that has not been used previously. Hence, results cannot be compared with other studies.

Wider implications of the findings: Relugolix-CT not only reduced HMB in women with UF, but also improved patient-reported outcomes, by reducing the distress caused by frequent UF symptoms and improving daily activities. Responder rates for both patient-reported outcomes provide meaningful results that are easy to interpret and communicate to patients.

Trial registration number: NCT03049735 and NCT03103087

O-025 Levonorgestrel-releasing intrauterine system (LNG-IUS) versus oral medroxyprogesterone acetate (MPA) in infertile women with endometrial hyperplasia without atypia: prospective evaluation of regression rates and live-birth rates

V.S. Vanni[1], G. Campo[1], E. Papaleo[2], F. Turba[1], D. Casiero[1], M. Sajdak[2], R. Villanacci[1], L. Quaranta[2], F. Pasi[2], M. Scarrone[3], S. Bordoli[3], V. Canti[3], M. Candiani[1], P. Rovere-Querini[3], P. Giardina[2]

[1]Università Vita-Salute San Raffaele, Obstetrics and Gynecology Dept., Milano, Italy ; [2]IRCCS Ospedale San Raffaele, Obstetrics and Gynecology Dept., Milan, Italy ; [3]Università Vita-Salute San Raffaele, Internal and Specialistic Medicine Dept., Milano, Italy

Study question: What is the role of Levonorgestrel-releasing intrauterine system (LNG-IUS) in the treatment of infertile women with endometrial hyperplasia without atypia (EH)?

Summary answer: EH regression rates are higher in patients receiving LNG-IUS compared to oral MPA. Live-birth rates following assisted reproduction treatments are comparable between the two groups.

What is known already: LNG-IUS is considered superior to continuous oral progestin therapy for treatment of EH in the general population (El Behery et al., 2015). Treatment of EH in infertile patients undergoing assisted reproduction treatments (ART) however requires special considerations, due to the need to preserve short-term endometrial receptivity. LNG-IUS causes downregulation of endometrial receptors to an extent that is greater than that observed after oral progesterone administration (Vereide et al., 2005). Thus, a detrimental effect of LNG-IUS on fertility outcomes could be hypothesized and should be excluded before widely adopting the use of LNG-IUS in the treatment of EH in infertile patients.

Study design, size, duration: This prospective cohort study was designed to evaluate the role of LNG-IUS as a first or second-line treatment for EH occurring in infertile patients undergoing ART. N=221 infertile women with a diagnosis of EH treated between December 2014 and April 2018 at the Reproductive Medicine Unit of the San Raffaele Hospital (Milan, Italy) were included. After EH regression, patients were admitted to ART.

Participants/materials, setting, methods: EH was diagnosed on hysteroscopic endometrial biopsies. First-line treatment was either oral MPA 10 mg daily (n=193) or LNG-IUS (n=28). In case of EH persistence, patients receiving oral MPA either switched to LNG-IUS (n=63) or continued daily MPA (n=14). Follow up biopsies were scheduled after 90 days of treatment, both for first and second line therapy. If LNG-IUS was used, it was kept in place during biopsy and removed after confirmation of negative histology.

Main results and the role of chance: Baseline characteristics and possible confounders such as age at diagnosis, BMI, parity, smoking status and duration of infertility did not differ between patients receiving LNG-IUS versus MPA as first-line treatment for EH (data not shown). EH regression rate at first follow up (3 months) was higher in the LNG-IUS group compared to the oral progestins group (28/28 and 116/193 respectively, 1000% vs 60.1%, p< 0.001). EH regression after second-line treatment was 61/63 in the group switching from MPA to LNG-IUS and 12/14 in the group continuing MPA (96.8 vs 85.7%, p=0.15). Overall, n=91 patients used LNG-IUS as either first or second-line treatment for EH, while n=130 only used MPA. The cumulative live-birth rate following ART in patients ever receiving LNG-IUS (21/91, 25.4%) was similar to the cumulative live-birth rate patients observed in patients only receiving MPA (33/130, 23.1%, p=0.75). Confounders including age at diagnosis (36.8 ± 1.8 vs 37.9 ± 0.3, Mean ± SD), BMI (23.1 ± 0.4 vs 22.1 ± 0.2, Mean ± SD) and number of previous failed ART cycles (1.3 ± 0.2 vs 1.8 ± 0.2, Mean ± SD) did not differ between patients ever receiving LNG-IUS versus patients only receiving MPA respectively.

Limitations, reasons for caution: Limitations of this study are the relatively small number of patients included and the fact that treatment's choice was not based on randomization but rather on patient's preference after thorough counselling. Another relevant information that is currently missing is a cost-analysis comparing the two groups.

Wider implications of the findings: LNG-IUS should be the preferred therapy for EH without atypia in infertile patients, as it provides very high curing rates already after 3 months of treatment and does not impair endometrial receptivity in the following ART cycles. Further prospective data collection and studies confirming our results will be of interest.

Trial registration number: na

O-026 Endometrial cancer risk in women with histological proven endometriosis or adenomyosis; a retrospective nationwide cohort study.

M. Hermens[1], I. Velthuis[2], R. Bekkers[1], H. Van Vliet[1], S. Bert[3], A. Van Altena[2]

[1]Catharina Hospital, Obstetrics and Gynaecology, Eindhoven, The Netherlands ; [2]Radboudumc, Obstetrics and gynaecology, Nijmegen, The Netherlands ; [3]Radboudumc, Pathology, Nijmegen, The Netherlands

Study question: What is the association between histological proven endometriosis/adenomyosis and endometrial cancer?

Summary answer: Women with histological proven endometriosis/adenomyosis have an increased risk of endometrial cancer.

What is known already: Women with histological proven endometriosis/adenomyosis have an increased risk of ovarian cancer. Smaller studies show conflicting results on the endometrial cancer risk in women with histological proven endometriosis/adenomyosis.

Study design, size, duration: A population-based retrospective cohort study of 131,646 women with histologically proven endometriosis/adenomyosis, matched with 132,700 women with a benign dermal nevus selected from the Dutch nationwide registry of histopathology and cytopathology (PALGA) between January 1990 and July 2017. In the endometriosis/adenomyosis group 1,820 (1.4%) women had histological reports on endometrial cancer and in the nevus group 771 (0.6%) women. Crude and age-adjusted incidence rate ratios (IRR) for endometrial cancer and its subtypes were estimated.

Participants/materials, setting, methods: We selected all women with histological proven endometriosis/adenomyosis and women with a benign dermal nevus diagnosed between 1990 and 2015 from the Dutch nationwide registry of histopathology and cytopathology.

Main results and the role of chance: The age-adjusted IRR for endometrial cancer overall was 29.60 (95%CI 26.40-33.18). Age at endometrial cancer diagnosis was similar for the endometriosis/adenomyosis and nevus group, 61 (IQR 55-69) and 62 (IQR 56-68), p=0.28, respectively.

After excluding the first year of follow-up the age-adjusted IRR was 1.19 (95%CI 0.94-1.52), indicating that endometrial cancer is most often found at time of histological diagnosis of endometriosis/adenomyosis. In 25.2% of the endometrial cancer cases in the endometriosis/adenomyosis group, the endometrial cancer diagnosis was not made until after hysterectomy. These women had not had prior (micro)curettage or biopsy.

Limitations, reasons for caution: These results are limited to women with histological proven endometriosis/adenomyosis, and prone to detection bias. Furthermore, no clinical information is available on possible confounders.

Wider implications of the findings: This study shows an association between endometriosis/adenomyosis and endometrial cancer. In most women, diagnosis of endometrial cancer and endometriosis/adenomyosis is at time of hysterectomy. To develop preventive strategies future studies should focus on the detection of women at risk for endometrial cancer in the group of women suspect for endometriosis/adenomyosis.

Trial registration number: not applicable

O-027 Efficacy and safety of linzagolix on heavy menstrual bleeding (HMB) due to uterine fibroids (UF): Results from a placebo-controlled, randomized, Phase 3 trial

H. Taylor[1], J. Donnez[2], R. Taylor[3], E. Bestel[4], J.P. Gotteland[5], A. Humberstone[4], E. Garner[6]

[1]Yale University- School of Medicine, Dept. of Reproductive Endocrinology and Infertility, New Haven- CT, U.S.A. ;
[2]Catholic University of Louvain, Société de recherche pour l'infertilité, Brussels, Belgium ;
[3]University of Utah Health, Department of Obstetrics and Gynecology, Salt Lake City, U.S.A. ;
[4]ObsEva SA, Clinical, Geneva, Switzerland ;
[5]ObsEva SA, Research and Development, Geneva, Switzerland ;
[6]ObsEva SA, Clinical, Boston, U.S.A.

Study question: Do doses of 100 or 200 mg linzagolix with or without estradiol 1 mg/NETA 0.5 mg add-back therapy (ABT) reduce HMB in women with UF?

Summary answer: Once daily oral doses of linzagolix 100 and 200 mg with and without ABT significantly reduced HMB compared to placebo after 24 weeks of treatment.

What is known already: Suppression of estradiol (E2) using GnRH analogues has been shown to be an effective treatment for UF-associated HMB. Full E2 suppression to postmenopausal levels is highly efficacious but requires hormonal ABT to prevent hypoestrogenic symptoms and bone mineral density (BMD) loss. Linzagolix is an investigational, oral GnRH receptor antagonist, which dose-dependently reduces E2 levels, providing full suppression (serum E2 < 20 pg/mL) and partial suppression with once daily oral dosing of 200 mg and 100 mg, respectively. We hypothesized that partial E2 suppression could reduce HMB without the marked BMD loss observed with full suppression.

Study design, size, duration: PRIMROSE 2 is a Phase 3, double-blind, randomized, placebo-controlled, multicenter trial evaluating 100 or 200 mg linzagolix with or without ABT for 52 weeks. 535 patients were randomized and equally distributed among treatment groups. Subjects randomized to placebo were crossed-over to 200 mg linzagolix + ABT after 24 weeks. Individual active *vs* placebo efficacy comparisons were conducted at 24 weeks using a 0.0125 significance level to account for multiplicity of four active treatment groups.

Participants/materials, setting, methods: Participants had HMB (> 80 mL menstrual blood loss (MBL)/cycle). The primary efficacy endpoint was HMB reduction at 24 weeks; responders were defined as having MBL (alkaline hematin method) of ≤ 80 mL and ≥ 50% reduction from baseline. Other assessments included amenorrhea, pain, uterine and fibroid volume, haemoglobin levels, and quality of life. E2 levels were measured. BMD was assessed centrally using Dual Energy X-ray Absorptiometry. Calcium/vitamin D were not provided or recommended.

Main results and the role of chance: Subjects had a mean age of 43 years and 218 mL baseline MBL; 5% were Black. At 24 weeks, responder rates were significantly higher (p<0.001) in all linzagolix groups compared to placebo (29.4%); 93.9%, 77.7%. 77.2% and 56.7% in the 200 mg with ABT, 200 mg without ABT, 100 mg with ABT and 100 mg without ABT groups, respectively. Amenorrhea rates were 80.6%, 70.9%, 63.4%, and 34.0% in the corresponding groups, compared to 11.8% for placebo (p<0.0125). All linzagolix treatments significantly (p<0.0125) reduced pain and uterine volume, and significant (p<0.0125) reduction in fibroid volume was observed for 200 mg linzagolix with and without ABT. Median serum estradiol levels were suppressed below 20 pg/mL in the 200 mg linzagolix without ABT group and were maintained from 20 to 60 pg/mL in the other groups.

Mean percent (95% CI) lumbar spine BMD changes from baseline to week 24 in the placebo, 100 mg, 100 mg + ABT, 200 mg and 200 mg + ABT dose groups were: 0.514% (-0.019;1.048), -2.068% (-2.630;-1.506), -0.992% (-1.572;-0.412), -4.019% (-4.668;-3.371), -1.305 (-1.923;-0.687), respectively. Changes in femoral neck and total hip BMD showed a similar pattern but were generally smaller.

Limitations, reasons for caution: This Phase 3 trial of linzagolix for UF-associated HMB included a relatively narrow population of primarily Caucasian European patients treated for 52 weeks. Post-treatment follow-up will provide more information on symptom recurrence and BMD recovery. Effects on BMD of calcium/vitamin D supplementation with linzagolix in UF are not known.

Wider implications of the findings: Two linzagolix doses were identified for treatment of UF-associated HMB: 200 mg with ABT, with a responder rate of 94%, and 100 mg without ABT, which has the potential for long-term treatment without ABT. Data from a similar ongoing US trial should provide information on linzagolix in a broader population.

Trial registration number: NCT03070951

SELECTED ORAL COMMUNICATIONS

SESSION 06: FROZEN VERSUS FRESH EMBRYO TRANSFER. AN ONGOING CHALLENGE ON CHILDREN'S HEALTH

06 July 2020 Parallel 5 10:00 - 11:30

O-028 Cardiac remodeling in fetuses conceived by assisted reproductive technologies following fresh versus frozen embryo transfer

M.L. Boutet[1], G. Casals[1], B. Valenzuela-Alcaraz[1], L. García-Otero[1], F. Crovetto[1], E. Vidal[1], D. Manau[1], E. Gratacos[1], F. Crispi[1]

[1]Barcelona Center for Maternal-Fetal and Neonatal Medicine Hospital Clínic and Hospital Sant Joan de Déu- ICGON- IDIBAPS- Universitat de Barcelona- Barcelona-Spain, Institut Clínic de Ginecologia- Obstetrícia i Neonatologia, Barcelona, Spain

Study question: Do fetuses conceived after frozen embryo transfer (FET) present signs of cardiac remodeling and dysfunction similar to those observed in fresh embryo-transfer (ET)?

Summary answer: Both fresh ET and FET present signs of fetal cardiac remodeling and dysfunction, with more pronounced changes in the fresh ET as compared to FET.

What is known already: We and others have previously demonstrated that fetuses and children conceived by assisted reproductive technologies present cardiac remodeling and dysfunction (Scherrer et al. Circulation 2012, Valenzuela-Alcaraz et al. Circulation 2013, Valenzuela-Alcaraz et al. BJOG 2018). These fetuses show more globular hearts, dilated atria, reduced longitudinal motion and impaired relaxation. Cardiac changes were already present in utero and persisted after birth. However, 90% of the fetuses included in these studies were conceived by in vitro fertilization (IVF) after fresh ET. It is unknown whether fetal cardiac remodeling is also present in FET.

Study design, size, duration: Prospective cohort study of 397 singleton pregnancies recruited from December 2010 to December 2019, including 132 spontaneously conceived (SC) pregnancies, 116 conceived by IVF following FET and 149 IVF after fresh ET. Fetal structural and functional echocardiography was performed in all pregnancies.

Participants/materials, setting, methods: Pregnancies conceived by IVF were recruited from a single Assisted Reproductive center, ensuring high homogeneity in IVF stimulation protocols, endometrial preparation for FET, laboratory procedures and embryo culture conditions. SC pregnancies from fertile couples (obtained after a period no longer than 12 months) were randomly selected from low-risk population and paired to IVF pregnancies by maternal age. Fetal

echocardiography was performed at 29-33 weeks of pregnancy to assess cardiac structure and function in all pregnancies.

Main results and the role of chance: Parental age, ethnicity, body mass index and smoking were similar among study groups. Median gestational age at echocardiography (29-33 weeks) and estimated fetal weight (1400-2000g) were similar in all study groups. Both fresh ET and FET groups showed significant signs of fetal cardiac remodeling and dysfunction, with more pronounced changes in the fresh ET as compared to FET. IVF fetuses showed larger atria (right atria-to-heart ratio: fresh ET median 18.1% [SD 18.1-18.2] vs FET 17.7% [17.6-17.8] vs SC 17.1% [17.1-17.2]; linear tendency P-value <0.001), more globular ventricles (right ventricular sphericity index: fresh ET 1.60 [1.58-1.62] vs FET 1.62 [1.60-1.64] vs SC 1.68 [1.66-1.70]; <0.001) and thicker myocardial walls (relative wall thickness: fresh ET 0.80 [0.78-0.81] vs FET 0.76 [0.73-0.77] vs SC 0.65 [0.63-0.66]; <0.001) as compared to SC pregnancies. Both fresh ET and FET groups had also signs of systolic and diastolic dysfunction with reduced left shortening fraction (fresh ET 36% [35.9-36] vs FET 37.1% [37-37.2] vs SC 37.3 [37.1-37.3]; <0.001) and increased left myocardial performance index (fresh ET 1.58 zscores [1.54-1.61] vs FET 1.54 [1.49-1.57] vs SC 1.30 [1.27-1.32]; <0.001) as compared to SC pregnancies. All differences remained statistically significant after adjustment by birthweight centile, preeclampsia and prematurity.

Limitations, reasons for caution: The cardiac changes reported here are subclinical, with most cardiovascular indexes lying within normal ranges.

These milder features in FET fetuses cannot condition the technique´s choice and must be considered together with the global perinatal results related to these gestations.

Wider implications of the findings: Although these features are recognized as potential cardiovascular risk factors, their association with adult cardiovascular disease remains to be proven. Identification of cardiac remodeling in these fetuses represent an opportunity to recognize them as potential high-risk population that might benefit from early preventive measures to improve their future cardiovascular health.

Trial registration number: not applicable

O-029 Maternal and treatment contributions to perinatal outcomes after transfer of fresh and cryopreserved embryos in assisted reproduction: A Nordic sibling study

K. Westvik-Johari[1], L.B. Romundstad[2], C. Bergh[3], M. Gissler[4], A.K.A. Henningsen[5], S.E. Håberg[6], U.B. Wennerholm[3], A. Tiitinen[7], A.B. Pinborg[8], S. Opdahl[9]

[1]Department of Public Health and Nursing-, Norwegian University of Science and Technology- Trondheim- Norway, Trondheim, Norway ;
[2]Norwegian Institute of Public Health- Oslo- Norway, Center for Fertility and Health, Oslo, Norway ;
[3]Institute of Clinical Sciences- Sahlgrenska Academy- University of Gothenburg- Sahlgrenska University Hospital, Department of Obstetrics and Gynaecology, Gothenburg, Sweden ;
[4]Statistics and Registers Unit, THL Finnish Institute for Health and Welfare, Helsinki, Finland ;
[5]Copenhagen University Hospital- Rigshospitalet, The Fertility Clinic, Copenhagen, Denmark ;
[6]Norwegian Institute of Public Health, Center for Fertility and Health, Oslo, Norway ;
[7]Helsinki University Hospital & University of Helsinki, Department of Obstetrics and Gynaecology, Helsinki, Finland ;
[8]Copenhagen University Hospital- Rigshospitalet, The Fertility Clinic, , Denmark ;
[9]Norwegian University of Science and Technology, Department of Public Health and Nursing, Trondheim, Norway

Study question: Are the adverse perinatal outcomes seen after transfer of fresh and cryopreserved embryos in assisted reproductive technology (ART) a consequence of maternal or treatment factors?

Summary answer: Fetal growth is more strongly influenced by treatment, whereas risk of preterm birth is more strongly influenced by maternal factors.

What is known already: Perinatal outcomes differ between children born after transfer of fresh (fresh-ET) and cryopreserved (cryo-ET) embryos. Fresh-ET children have lower birthweights, higher risk of small-for-gestational age (SGA) and preterm birth (PTB), while cryo-ET children have higher birthweights, risk of large-for-gestational age (LGA) and PTB, compared to spontaneously conceived children (SC). It is unknown to what extent these adverse outcomes are caused by parental or treatment factors.

Sibling comparisons help disentangle the parental and treatment contributions to perinatal health in ART children. Previous sibling studies show conflicting results, and most could not separate children born after fresh-ET and cryo-ET.

Study design, size, duration: Registry-based cohort study including liveborn singletons in the Committee of Nordic ART and Safety (CoNARTaS) cohort from Denmark (1994-2014), Norway (1988-2015) and Sweden (1988-2015). The population level analyses included 53,345 children born after fresh-ET, 14,405 after cryo-ET, and 2,563,837 spontaneously conceived (SC) children. In total, 27,041 maternal sibling groups with at least two different conception modes were identified.

Outcomes were birthweight, SGA, LGA, preterm birth (<37 weeks) and very preterm birth (VPTB, <32 weeks).

Participants/materials, setting, methods: Data from nationwide medical birth registries and ART registries/databases, were linked using unique national identity numbers. We compared perinatal outcomes according to treatment modes in multilevel linear and logistic models, where fixed effects (within sibling groups) were attributed to treatment. Fixed effects models included adjustment for birth year and maternal age, parity, smoking, and body mass index. For comparison, random effects models provided population estimates, additionally adjusted for country and maternal height.

Main results and the role of chance: Children born after cryo-ET had higher birthweights (mean difference 73 grams, 95% confidence interval [CI] 59 to 87) and increased risk of LGA (odds ratio [OR] 1.75, 95% CI 1.42-2.2) compared to their SC siblings. In contrast, children born after fresh-ET had lower birthweights (mean difference -56 grams, 95% CI -65 to -47) and increased risk of SGA (OR 1.31, 95% CI 1.13-1.52), compared to their SC siblings. These estimates were comparable to associations at the population level (random effects).

Fresh-ET showed the highest risk of PTB at the population level (OR 1.66, 95% CI 1.59-1.73), but risk was increased also for cryo-ET (OR 1.44, 95% CI 1.32-1.57). Comparing siblings, the association attenuated to a modest risk increase, similar for fresh-ET (OR 1.26, 95% CI 1.13-1.41) and cryo-ET (OR 1.23, 95% CI 1.03-1.48). VPTB showed stronger associations at the population level for both fresh-ET (OR 2.07, 95% CI 1.89-2.26) and cryo-ET (OR 1.70, 95% CI 1.40-2.04), which attenuated completely in the sibling comparisons (fresh-ET OR 1.04, 95% CI 0.81-1.34; cryo-ET OR 0.95, 95% CI 0.63-1.44).

Results were independent of which conception method was the first in each sibling group and remained similar when restricted to full siblings.

Limitations, reasons for caution: Although we could adjust for unmeasured, constant parental factors, we cannot exclude confounding from unmeasured, non-constant parental factors. Information on causes of infertility was limited. Consequently, we could not investigate if parental contribution depends on infertility causes. Further, our study cannot identify specific mechanisms underlying treatment and parental contributions.

Wider implications of the findings: Adverse perinatal health after ART can be attributed to a combination of parental and treatment factors. Risk of PTB is primarily caused by parental factors whearas fetal growth deviation (SGA in fresh-ET and LGA in cryo-ET) is primarily caused by treatment factors. Further investigations of responsible treatment factors are warranted.

Trial registration number: Not applicable

O-030 Birth weight and large-for-gestational-age in singletons born after frozen compared to fresh embryo transfer by gestational week at birth: a Nordic cohort study

A. Terho[1], S. Pelkonen[1], S. Opdahl[2], L.B. Romundstad[3], C. Bergh[4], U.B. Wennerholm[4], A.K.A. Henningsen[5], A. Pinborg[5], M. Gissler[6], A. Tiitinen[7]

[1]Oulu University Hospital- University of Oulu, Department of Obstetrics and Gynaecology, Oulu, Finland ;
[2]Norwegian University of Science and Technology, Department of Public Health and Nursing, Trondheim, Norway ;
[3]Norwegian Institute of Public Health, Center for Fertility and Health, Oslo, Norway ;
[4]Sahlgrenska Academy- University of Gothenburg- Sahlgrenska University Hospital, Department of Obstetrics and Gynaecology- Institute of Clinical Sciences, Gothenburg, Sweden ;

[5]Copenhagen University Hospital- Rigshospitalet, The Fertility Clinic, Copenhagen, Denmark ;
[6]THL- Finnish Institute for Health and Welfare, Statistics and Registers Unit, Helsinki, Finland ;
[7]University of Helsinki, Department of Obstetrics and Gynaecology, Helsinki, Finland

Study question: When does the intrauterine growth difference become apparent between singletons born after frozen embryo transfer (FET) and fresh embryo transfer (fresh ET)?
Summary answer: Mean birth weights and proportion of large-for-gestational-age (LGA) become significantly higher among FET singletons starting from gestational week (GW) 33 and 36, respectively.
What is known already: In recent years there has been a steep rise in recorded FET treatments, enabling widespread use of elective single embryo transfer, thus reducing the risks associated with multiple gestations. However, FET singletons are heavier and there is a higher risk of LGA (birth weight > 90 percentiles) compared to fresh ET singletons. In turn, risk of small-for-gestational-age (SGA, birth weight < 10 percentiles) is lower in FET singletons compared to fresh ET singletons. The reasons, timing and consequences of these differences remain largely unclear. There is limited evidence that this difference in growth develops by the last trimester of pregnancy.
Study design, size, duration: A retrospective Nordic register-based cohort comparing singletons born after FET (*n* = 17 500) to singletons born after fresh ET (*n* = 69 510) and spontaneous conception (SC) (*n* = 3 311 588). All live born singletons between the years 2000 and 2015 in Denmark, Norway and Sweden with gestational age ≥ 22 weeks at birth were included from the population-based Committee of Nordic ART (assisted reproductive technology) and Safety (CoNARTaS) study population.
Participants/materials, setting, methods: FET children were compared to fresh ET and SC for mean birth weight and proportion of LGA and SGA for each GW at birth. χ^2 test and test for relative proportions were used to compare categorical and Student's t-test to compare continuous variables. Adjusted odds ratios for LGA and SGA were calculated using logistic regressions, adjusting for year of birth, maternal age, parity, BMI, chronic hypertension, diabetes, smoking and offspring sex.
Main results and the role of chance: Mean birth weights were significantly higher for FET boys compared to fresh ET boys starting from GW 33 and for FET girls starting from GW 34 (range from p<0.001 to p=0.046 by week). FET boys had a significantly higher proportion of LGA (11.0-15.1%) at birth between GW 36-42 compared to fresh ET (7.1-9.4%) (range from p<0.001 to p=0.048 by week). For FET girls this was seen between GW 37-42 (10.6-13.4%) compared to fresh ET (6.6-8.0%) (range from p<0.001 to p=0.009 by week).

The proportion of SGA was lower among FET boys (7.6-8.7%) compared to fresh ET (11.9-13.6%) between GW 36-42 (range from p<0.001 to p=0.016 by week). For FET girls this was seen between GW 38-42 (7.0-9.3%), compared to fresh ET (13.0-14.6%) (p<0.001).

The rate of LGA was significantly higher for FET boys between GW 38-41 (p<0.001) and for FET girls between GW 37-40 (range from p<0.001 to p=0.018 by week) compared to SC children.

All singletons born after FET had a higher risk of LGA compared to singletons born after fresh ET (adjusted odds ratio (aOR) 1.87, 95% confidence interval (95% CI) 1.76 - 1.98) and singletons born after SC (aOR 1.28, 95% CI 1.22 - 1.35).
Limitations, reasons for caution: There may be residual confounding factors that we were not able to control for, most importantly the causes of preterm birth, which may also have an influence on the fetal growth. Also, the number of children born extremely preterm or post-term is limited even in a large study design.
Wider implications of the findings: This is, to date, the largest study on birth weights among preterm and term ART singletons with a population-based design and SC control group. Growth differences are an important aspect of the safety profile of ART. More research is needed on the long-term outcome of these children.
Trial registration number: not applicable

O-031 Development of children born from freeze-only versus fresh embryo transfer: follow-up of a randomized controlled trial

T.N.L. Vuong[1], T. Ly[2], N. Nguyen[3], L. Nguyen[4], X. Le[2], T. Le[2], K. Le[2], T. Le[2], M. Nguyen[4], V. Dang[2], R. Norman[5], B. Mol[6], T. Ho[4]

[1]University of Medicine and Pharmacy at Ho Chi Minh City, Department of Obstetrics and Gynecology, Ho Chi Minh City, Vietnam ;
[2]My Duc Hospital, IVFMD- HOPE Research Center, Ho Chi Minh City, Vietnam ;
[3]University of Medicine and Pharmacy at Ho Chi Minh City, Department of Pediatrics, Ho Chi Minh City, Vietnam ;
[4]My Duc Hospital, HOPE Research Center, Ho Chi Minh City, Vietnam ;
[5]University of Adelaide, Fertility SA, Adelaide, Australia ;
[6]School of Medicine- Monash University, Department of Obstetrics and Gynecoloy, Melbourne, Australia

Study question: What are the long-term development outcomes in children born after freeze-only versus fresh embryo transfer (ET) in women without polycystic ovary syndrome (PCOS)?
Summary answer: Children born from non-PCOS women undergoing frozen ET had better problem solving and possibly better fine motor skills than children born from fresh ET.
What is known already: A recent randomized controlled trial (RCT) showed comparable ongoing pregnancy and live birth rates after use of a freeze-only versus fresh ET strategy in non-PCOS women undergoing IVF/ICSI (NCT02471573; Vuong LN, et al. NEJM 2018;378:137-147). Birth weight was significantly higher in children born from frozen ET compared with fresh ET. Here, we report on the development of babies born after frozen versus fresh ET.
Study design, size, duration: This was a long-term follow-up study (NCT04099784) of babies born alive after the first ET in the original RCT. Of 391 couples randomised, live birth occurred in 132 (97 singleton/35 twins) and 123 (95 singleton/28 twins) women in the freeze-only and fresh ET group, respectively; 113 parents (86%) from the freeze-only group (147 babies) and 99 (80%) from the fresh ET group (120 babies) returned questionnaires for this follow-up study.
Participants/materials, setting, methods: At least 2 years after birth, parents of all babies born alive from the first transfer after freeze-only or fresh ET in the RCT were contacted via postal mail and e-mail and asked to complete and return the Developmental Red Flags and Ages & Stages Third Edition (ASQ-3) Questionnaires. Parents received training and completed questionnaires at home, and returned questionnaires via postal mail, e-mail or instant messenger.
Main results and the role of chance: The mean age of children at the end of follow-up was 37 months. Height (95.0±6.1 vs 95.7±5.6 cm; p=0.382) and weight (14.9±2.6 vs 14.8±2.6 kg; p=0.903) were comparable in the freeze-only and fresh ET groups (results were also similar when singletons and twins were analysed separately). ASQ-3 scores for problem solving were significantly better in the freeze-only versus fresh ET group (overall: 53.6±8.4 vs 51.1±10.2, p=0.041; singletons: 52.3±10.1 vs 51.0±9.9, p=0.776; twins: 55.0±5.5 vs 51.4±11.1; p=0.202), and there was at trend towards higher fine motor skills scores in the freeze-only versus fresh ET group (overall: 47.8±11.6 vs 44.9±12.6; p=0.056; singletons: 46.0±13.4 vs 45.4±12.6, p=0.992; twins: 49.85 ± 8.72 vs 43.93 ± 12.71, p=0.060). The overall proportion of children with abnormal ASQ-3 (6.8% vs 8.3%) or abnormal Red Flags (5.4% vs 6.7%) findings in the freeze-only and fresh ET groups was low and did not differ significantly between groups. In a quartile-based analysis including predictive variables for ASQ-3, there was no significant association between estradiol or progesterone concentrations and ASQ-3 overall or problem solving scores.
Limitations, reasons for caution: This analysis includes only babies born alive after IVF/ICSI, and not all parents from the original study returned the study questionnaires.
Wider implications of the findings: Use of a freeze-only strategy resulted in better problem solving and possibly better fine motor skills scores than fresh embryo transfer, with comparable results in other domains. This highlights the importance of evaluation of normal development in children born after fresh compared with frozen embryo transfer.
Trial registration number: NCT04099784

O-032 Health in childhood following assisted reproductive technology (HiCART) - an ongoing study of 600 children born after assisted reproductive technology (ART) in Denmark

L.L. Asserhøj[1,2], A.S.L. Kjaer[1,2], I. Mizrak[1], G.F. Heldarskard[1], T.D. Clausen[3], G. Greisen[4], K.M. Main[2], P.L. Madsen[5,6], R.B. Jensen[2], A. Pinborg[1]

[1]Rigshospitalet, Fertility Clinic, Copenhagen, Denmark ;
[2]Rigshospitalet, Department of Growth and Reproduction, Copenhagen, Denmark ;

[3]Nordsjællands Hospital- Hillerød, Department of Obstetrics and Gynecology, Hillerød, Denmark ;
[4]Rigshospitalet, Department of Neonatology, Copenhagen, Denmark ;
[5]Herlev-Gentofte Hospital, Department of Cardiology, Copenhagen, Denmark ;
[6]Copenhagen University, Department of Clinical Medicine, Copenhagen, Denmark

Study question: Is body composition of children born after frozen embryo transfer (FET) different compared to children born after fresh embryo transfer and spontaneously conceived (SC) children?
Summary answer: Inclusion is ongoing, but preliminary results revealed an increased fat-percentage in the cohort despite a BMI close to mean according to a normal reference material.
What is known already: Children conceived after FET are at increased risk of being born large for gestational age (LGA) compared with children conceived after fresh embryo transfer and SC children, whereas children conceived after fresh embryo transfer have an increased risk of being born preterm and small for gestational age (SGA) compared with SC children. Studies suggest that ART may induce epigenetic variations around implantation, fertilization and at the early embryonic stages, but the potential long-term effects of ART on the health of the offspring and the underlying mechanisms are scarcely explored.
Study design, size, duration: This cohort study consists of 600 pre-pubertal singletons born after FET (n=200), fresh embryo transfer (n=200) or spontaneous conception (n=200) in Denmark from 2009-2012. All children are identified by their mother's personal identification number in the national ART and medical birth registry. The recruitment and examination started January 2019 and is expected to be completed in September 2020. Currently, 289 children (134 boys, 155 girls) have been examined.
Participants/materials, setting, methods: The children (age 6-9 years) undergo a clinical examination with anthropometric measurements including body mass index (BMI), whole body DXA-scan, blood pressure, pubertal staging and blood sampling (e.g. glucose profile, lipid profile, calcium homeostasis, hormonal levels (e.g. IGF-I, IGFBP-3, androgen status, AMH) genetic and epigenetic analyses). Both parents fill in a questionnaire regarding the pregnancy, medical history and current health.Maternal, obstetric and neonatal medical data are obtained from the national ART and birth registry.
Main results and the role of chance: Children born after FET had higher birth weight (SDS) compared to children born after fresh embryo transfer and SC children (FET vs. fresh embryo transfer: 0.31 [SD 0.96] vs. -0.20 [SD 1.05], p=0.001, FET vs. SC: 0.31 [SD 0.96] vs. -0.11 [SD 0.97], p=0.006). Mothers conceiving after ART were older (FET vs. SC: 35.1 vs. 32.2 years, p<0.001, fresh embryo transfer vs SC: 34.2 vs. 32.2 years, p=0.002) and of lower parity compared to mothers conceiving spontaneously (FET vs SC: 1.50 vs. 1.76, p=0.02, fresh embryo transfer vs. SC 1.28 vs. 1.76, p<0.001). There were no differences regarding pregestational BMI and gestational age.To preserve blinding and avoid false preliminary conclusions results are presented only for girls and boys respectively. Height (SDS) was 0.19 [SD 1.04] in boys and 0.16 [SD 1.02] in girls. Weight (SDS) was 0.09 [SD 1.07] in boys and 0.10 [SD 0.99] in girls. BMI (SDS) was 0.01 [SD 1.11] in boys and 0.04 [SD 1.02] in girls. These results are in accordance with Danish sex-matched references. DXA-scans showed a fat-percentage (SDS) of 1.70 [SD 0.78] in boys and 1.06 [SD 0.69] in girls which was markedly higher for boys compared to reference material.
Limitations, reasons for caution: The study is powered to detect a difference of 0.3 SD in BMI (primary endpoint). Thus, smaller differences may be overlooked. Other outcomes may be relevant, but analyses are exploratory by definition. Mothers included in the two ART groups may have different infertility background which may bias the results.
Wider implications of the findings: This study will provide important information on the health of children born after ART that may influence the choices made early in the planning of a fertility treatment. The study has the potential to reduce some of the uncertainties associated with the long-term consequences of ART.
Trial registration number: ClinicalTrials.gov identifier: NCT03719703

O-033 Do assisted reproductive treatments impact the risk of congenital anomalies in singletons? A longitudinal national French study

J. De Mouzon[1], P. Fauque[2], G. Viot[3], A. Devaux[4], F. Pessione[5], A.W.G. ART women and children health[6]

[1]Epidemiology, Infertility, Paris, France ;
[2]Université Bourgogne Franche-Comté - INSERM UMR1231, Biologie de la Reproduction, Dijon, France ;
[3]Clinique de La Muette, Unité de Génétique, Paris, France ;
[4]CHU d'Amiens, Centre d'assistance médicale à la procréation- biologie de la reproduction, Amiens, France ;
[5]Agence de la biomédecine, Pole Evaluation Biostatistique, La Plaine Saint Denis, France ;
[6]Agence de la Biomedecine, Working group ART women and children health, La Plaine Saint Denis, France

Study question: Do some specific assisted reproductive treatments (ART) present a higher risk of congenital anomalies in singletons?
Summary answer: After multivariate adjustments, including female infertilities, the increased risks of congenital defects associated with intra-uterine insemination were no longer significant, they only subsisted in IVF-group.
What is known already: Many epidemiological studies suggest that singletons born from assisted reproductive technologies (ART) have a higher risk of birth defects, specifically for musculoskeletal, cardiovascular and urogenital disorders. However, most of these studies were established on data at birth or in neonatal period and from relatively small population or from several registries. Moreover, to our knowledge, the female infertility, a major potential confounder, was not included into the risk calculation.
Study design, size, duration: Using data from the French National System database (SNDS), we conducted a comparative cohort study on all singleton births (deliveries ≥22 weeks of gestation and/or >500g of birthweight) that occurred in France over a 5-year period (2013-2017) resulting from fresh embryo or frozen embryo transfers (fresh-ET or FET from IVF/ICSI cycles), intrauterine insemination (IUI) and natural conceptions (NC). Data was available for this cohort of children at least up to early childhood (2.5 years old).
Participants/materials, setting, methods: A total of 3,501,496 singleton births were included (including 20,218 from IUI, 45,303 from fresh-ET; 18,885 from FET). Data were extracted from national health databases. We monitored the major birth defects. Malformations were classified according to the International Classification of Disease (ICD-10). To analyse the effect of ART conception, multivariate analyses were performed with multiple logistic regression models adjusted for maternal age, primiparity, obesity, smoking, history of high blood pressure or diabetes and female infertility.
Main results and the role of chance: In our cohort of children, the whole prevalence of congenital malformations was 3.8%: 3.8% after NC, 4.5% after fresh-ET, 4.4% after FET, and 3.9% after IUI. Compared with infants conceived naturally, children born after fresh-ET and after FET had a significantly higher prevalence of malformations, with an aOR of 1.15 [95%CI 1.10–1.21, p<0.0001] and aOR of 1.12 [95%CI 1.05–1.21, p=0.001], respectively. Among the fifteen relevant subgroup of malformations studied, we observed a significant increased risk for eight of them in the fresh-ET group compared with the NC group (i.e. cleft lip and/or palate: aOR 1.39 [95%CI 1.11–1.74, p=0.004], respiratory: aOR 1.38 [95%CI 1.08–1.78, p=0.012], musculoskeletal: aOR 1.19 [95%CI 1.09–1.30, p<0.0001], nervous: aOR 1.26 [95%CI 1.09–1.47, p=0.002], digestive: aOR 1.25 [95%CI 1.09–1.44, p=0.002], urinary: aOR 1.14 [95%CI 1.01–1.28, p=0.029], cardiovascular systems with namely heart defects: aOR 1.14 [95%CI 1.03–1.26, p=0.013]). In the FET group, this increased risk was observed for face (aOR 2.83 [95%CI 1.39–5.75, p=0.004]) and digestive systems (aOR 1.27 [95%CI 1.03–1.57, p=0.002]. The overall risk of congenital malformations was similar in the IUI group and the NC group, as well as for specific anomalies.
Limitations, reasons for caution: For some extremely rare malformations, the sample size may be too small to reliably conclude that there was no difference between groups. Male infertility, the *in vitro* fertilization method and embryo stage at transfer could not be taken into account in the analyses. Furthermore, residual confounding cannot be excluded.
Wider implications of the findings: In this large study, after multivariate maternal adjustments the increased risks of children defects subsisted after IVF but relatively moderate, and those associated with IUI were no longer significant. These findings highlight that underlying parental infertility could contribute to the increased risk of children defects associated with ART.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 07: MALE AND FEMALE FERTILITY PRESERVATION - CLINICAL ASPECTS

06 July 2020 Parallel 6 09:50 - 11:40

O-034 Delaying cryopreservation for up to 48 hours does not adversely affect ovarian tissue used for fertility preservation

B. Bjarkadottir[1], M. Zemyarska[1], X. Wei[1], C. Walker[1], S. Lane[2], J. Davies[3], S. Williams[1]

[1]University of Oxford, Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom ;
[2]Children's Hospital Oxford. Oxford University Hospitals NHS Foundation Trust, Department of Paediatric Oncology and Haematology, Oxford, United Kingdom ;
[3]Children's Hospital Oxford- Oxford University Hospitals NHS Foundation Trust, Oxford Cell and Tissue Biobank, Oxford, United Kingdom

Study question: Does delaying processing and cryopreservation of ovarian tissue by 24 or 48 hours adversely affect primordial follicles?
Summary answer: A 24 or 48 hour delay before cryopreservation of ovarian tissue does not negatively impact primordial follicle morphology nor follicle growth after 10-day xenotransplantation.
What is known already: Ovarian tissue cryopreservation is becoming an increasingly common method of fertility preservation. While in most cases the tissue is processed and cryopreserved immediately upon procurement, delaying processing for up to 24 or even 48 hours could help with workload management. In addition, ovaries may be procured at a satellite site and transported to a cryopreservation facility, which necessitates a delay in processing. Little is known about the effects delayed processing may have on primordial follicle health and the functional potential of the tissue.
Study design, size, duration: Lamb ovaries (n=6 pairs of ovaries) were used to model human ovarian tissue. One ovary per pair was either processed into cortical strips immediately or stored whole at 4°C for 24 or 48 h before processing. Cortical strips were fixed fresh or after cryopreservation and thawing. Cryopreserved-thawed tissue (immediate processing, 24 or 48 h delay) was xenografted subcutaneously into immunocompromised mice (n=3) for 10 days to allow follicle development. Fixed samples were embedded and sectioned.
Participants/materials, setting, methods: Up to 50 primordial follicles from each cortical strip (one per condition/animal) stained with haematoxylin and eosin were classified by morphology as healthy or unhealthy. A proportional odds model was used to determine whether a delay in processing with or without cryopreservation affected follicle health. A logistic regression model was used to determine the odds of follicles being classified as growing after xenotransplantation. Data is presented as odds ratio (OR) with 95% confidence intervals (CI).
Main results and the role of chance: A processing delay of 24 h did not impact the health of primordial follicles in fresh (OR=0.78, 95%CI 0.38-1.60, $p>0.05$) and cryopreserved-thawed tissue (OR=0.93, 95% CI 0.61-1.41, $p>0.05$; no delay n=6; 299 fresh follicles, 287 cryopreserved follicles; 24 h delay n=3; 149 fresh follicles, 132 cryopreserved follicles). Conversely, a processing delay of 48 h (fresh tissue, n=3; 149 follicles) resulted in primordial follicles being more likely to be graded as unhealthy compared to immediately-processed tissue (OR=2.47, 95%CI 1.29-4.71, $p<0.01$). Interestingly however, tissue cryopreserved after a 48 h processing delay (n=3, 149 follicles) was less likely to contain unhealthy follicles compared to the immediately-processed cryopreserved control (OR=0.56, 95% CI 0.36-0.87, $p<0.01$). Xenografting of cryopreserved ovarian tissue after 0, 24 or 48 h processing delay demonstrated that grafts from all groups were functional, with no difference in the proportion of growing follicles between groups (immediate processing: 17.3±30.0%; 24 h delay: 46.0±2.8%; 48 h delay: 45.3±10.8%). Furthermore there was no difference in the health of primordial follicles in xenografted tissue after a 24 (OR=1.30, 95% CI 0.59-3.22, $p>0.05$) or 48 h delay (OR=1.26, 95% CI 0.55-3.96, $p>0.05$).
Limitations, reasons for caution: These findings should be validated using human ovarian tissue with a larger sample size to account for the natural variability between subjects. Ovaries were left intact prior to processing and the findings may therefore not be applicable to biopsied tissue. These results reflect follicle health and not follicle number.
Wider implications of the findings: These results demonstrate that ovaries can be stored for up to 48 h prior to cryopreservation with no adverse effect on primordial follicles after transplantation and *in vivo* development of the tissue. This may have implications on the way ovarian tissue cryopreservation is organised on a local and national level.
Trial registration number: N/A

O-035 Ovarian function recovery in newly diagnosed advanced Hodgkin lymphoma patients treated with PET-adapted regimen: prospective analysis of a multicentric randomized phase 3 trial (AHL2011).

I. Demeestere[1], J. Dechene[1], J. Racape[2], R. Bouabdallah[3], P. Brice[4], H. Ghesquieres[5], A. Stamatoulas[6], J. Dupuis[7], A.C. Gac[8], T. Gastinne[9], B. Joly[10], K. Bouabdallah[11], O. Casasnovas[12]

[1]Université Libre de Bruxelles, Research Laboratory on Human reproduction, Brussels, Belgium ;
[2]Université Libre de Bruxelles, Research Center in Epidemiology- School of public Health- and Biomedical Research Department of CUB-Erasme, Brussels, Belgium ;
[3]Institut P. Calmette, Department of hematology-, Marseille, France ;
[4]APHP- Hopital Saint Louis, Department of Hematology, Paris, France ;
[5]Hospices Civils de Lyon- Centre Hospitalier Lyon-Sud- et Université Claude Bernard Lyon-1, Department of hematology, Pierre Bénite, France ;
[6]Centre H. Becquerel, Department of hematology, Rouen, France ;
[7]H. Mondor Hospital, Department of hematology, Creteil, France ;
[8]Institut d'hématologie de basse normandie, Department of hematology, Caen, France ;
[9]University Hospital of Nantes, Department of hematology, Nantes, France ;
[10]Sud Francilien Hospital, Department of hematology, Corbeil-Essonnes, France ;
[11]CHU de Bordeaux, Department of Hematology, Bordeaux, France ;
[12]University Hospital F Mitterrand and Inserm UMR1231, Department of hematology, Dijon, France

Study question: Does de-escalated treatment strategy by switching regimen (BEACCOPP to ABVD) in early responder reduce the risk of ovarian damage and infertility compared to standard escalated BEACOPP regimen?
Summary answer: Although both treatments affected the ovarian reserve, de-escalated regimen decreased the risk of premature ovarian insufficiency compared to standard regimen in Hodgkin lymphoma patients.
What is known already: Chemotherapy-induced ovarian damage is a major concern for young patients treated for advanced Hodgkin lymphoma. Six cycles of bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP escalated) showed an improved progression-free survival rate compared to doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) in advanced stage diseases but induced high risk of immediate and long-term toxicities. AHL2011 trial recently demonstrated that the use of ABVD after two cycles of induction with BEACOPP in early responder based on PET-driven strategy was safe while minimizing toxicities (Casasnovas et al, Lancet Oncol 2019). Here we investigated its benefit on ovarian function in young patients.
Study design, size, duration: AHL2011 is a prospective randomized non inferiority trial enrolling 823 patients from May 19, 2011, to April 29, 2014 in 90 centers across Belgium and France. All female patients under 45-year old participating to AHL2011 trial were recruited for this prospective sub-analysis of the ovarian function and fertility at baseline, end of the treatment, and every year during 5 years of follow-up.
Participants/materials, setting, methods: Ovarian function was assessed by use of serum levels of FSH, estradiol and anti-mullerian hormone (AMH). All AMH measurements were centralized. Data on menstruation, use of oral contraceptive, fertility preservation, pregnancies and oncological outcomes were also collected. Acute premature ovarian insufficiency (POI) was defined as FSH value above 24 IU/L twice during 5-years follow-up when available, with estradiol below 50pg/ml, and low ovarian reserve by AMH value below 0.16ng/ml. Statistics were performed using STATA15.
Main results and the role of chance: A total of 146 patients were eligible for ovarian function assessment, 70 in the standard (BEACOPP escaladed) and 76 in the study (PET-driven strategy) groups. Mean age was similar between both groups (26.7±6.6 and 26.9±6.6 years old, respectively) as well as

oncological characteristics. As expected, the patients in the study group received significantly less cumulative doses of alkylating agents within a shorter timeframe. Ovarian function was similar between groups before treatment but became significantly different during follow-up. FSH values were significantly higher in the standard group while AMH values were lower. Thirty-two patients faced POI (46.1% versus 14.5% in standard and study groups, respectively). The risk of POI was significantly associated with age, total dose of alkylating agents, and groups with a lower risk in the study group (OR 0.20, 95% CI 0.08-0.50; p<0.001). Interestingly, low ovarian reserve was not associated with groups (OR 0.7, 95% CI 0.31-1.69; p=0.46). Although OR was always lower in the study group, association between low ovarian reserve and chemotherapy doses does not reach significance except for etoposide (OR 0.36, 95% CI 0.14-0.96; p<0.04 for doses <5000mg). A total of 29 and 30 patients reported pregnancies after treatment in standard and study groups, respectively (p=0.81).

Limitations, reasons for caution: Although this is the larger prospective study analyzing ovarian function in young advanced Hodgkin lymphoma patients considering a new therapeutic strategy, the number of patients remains limited and hormonal levels were not available at all time-points for all patients during the 5 years of follow-up.

Wider implications of the findings: The PET-driven treatment in young advanced Hodgkin lymphoma patients allows to reduce the risk of POI by 5 times compared to standard regimen. Although the ovarian reserve is also better preserved using PET-driven strategy, around 60% of the patients had a low AMH levels during the follow-up in both groups.

Trial registration number: NCT01358747

O-036 Live birth rate and utilization rate of eggs and embryos following fertility preservation (FP) in 879 female cancer patients over 19 years

D. Khalife[1], S. Ali[1], Y. Khalaf[1], N. Reddy[1], J. Kopeika[1]

[1]Guy's and St Thomas' NHS Foundation Trust, Assisted Conception Unit, London, United Kingdom

Study question: We aim to investigate the rate of women proceeding to FP at the time of cancer diagnosis, the return, utilization and live birth rates after cancer treatment.

Summary answer: Nearly half of newly diagnosed cancer patients proceed to FP and return for follow-up within 21 months post-cancer treatment. The livebirth rate (LBR) is 72.1%.

What is known already: FP is an established part of cancer services in many countries. It is used now more and more frequently. However, very little is published about long term utilization of gametes/embryos after FP. In male population of cancer patients concern is being raised that in spite of "routine" storage of sperm, less than 10% of patients return to use the stored gametes. Not much information is found for female cancer survivors.

Study design, size, duration: A prospective cohort study was conducted on 879 young women diagnosed with cancer who sought FP counseling at the Assisted Conception Unit (ACU) at Guy's and St Thomas' Hospital (GSTT), London, United Kingdom between January 2000 and December 2019.

Participants/materials, setting, methods: Data on 879 cancer patients were analyzed. Baseline characteristics include age, AMH, AFC, and cancer type. The primary outcome measure was total LBR. The secondary outcomes were return and utilization rates which were calculated as the number of patients who returned for follow-up and those who undergone embryo transfer. Means and frequencies were used to describe continuous and categorical variables respectively. Student t-test analysis was used with p <0.05 considered being statistically significant.

Main results and the role of chance: A total of 879 cancer patients received FP counseling at GSTT with breast cancer being the most common malignant disease accounting for 63.1% of the cases. The mean age, AMH, and BMI were 33.8 ± 7.8 years, 18.8 ± 20.5 pmol/L and 23.7 ± 4.2 kg/m^2 respectively. A total of 373 patients (42.4%) underwent FP of whom 40.7 % opted for embryo cryopreservation, 53.4% for oocyte cryopreservation, 5.1% had both and 0.76% opted for ovarian tissue cryopreservation in a different facility.

As for the return rate, 33.8% (297/879) of cancer patients returned for follow-up for assessment of ovarian function, menopausal symptoms, Hormone Replacement Therapy, and fertility treatment. Until today, utilization rate among those who had frozen gametes is16.4 % (61/373) and the overall LBR is 72.1% (44/61) of which 9.1% (4/44) are twin births. The miscarriage rate is 12.2% (8/61). The overall mean time to follow-up is 21.2 ± 19 months (range 1-132 months), with 66% of returning patients doing so within 2 years after cancer diagnosis.

Patients with breast cancer were more likely to return to use their gametes (27/61: 44.3%) and had significantly higher LBR (19/27: 70.3%) in comparison to patients with lymphoma (3/8: 37.5%) (p-value <0.001).

Limitations, reasons for caution: Although we are certain of capturing the outcome of those who had fertility treatment, we can't be as certain of capturing all births resulting from natural conception.

A proportion of patients may need a longer time to be able to attempt pregnancy, thus the calculated LBR can be underestimated.

Wider implications of the findings: This is a demonstration of how FP can be effective. Over nearly two decades of follow-up, 1 in 6 patients who underwent FP utilized their stored gametes/embryos with a good outcome. This is the first published account of the utilization rate after FP and the longest reported period.

Trial registration number: Not applicable

O-037 Long-term fertility and pregnancy outcomes in men and women with a history of childhood cancer: a nationwide population-based linkage analysis

M. Das[1], C. Wang[2], S. Oberg[3]

[1]Chelsea and Westminster Hospital NHS Foundation Trust, Reproductive Medicine- Obstetrics and Gynaecology, London, United Kingdom ; [2]Karolinska Institutet, Medical Epidemiology and Biostatistics, Stockholm, Sweden ; [3]Karolinska Institutet- Harvard T.H.Chan School of Public Health, Medical Epidemiology and Biostatistics, Stockholm, Sweden

Study question: What are the long-term fertility and pregnancy outcomes in men and women with a history of childhood cancer as compared to the general population?

Summary answer: Childhood cancer survivors are less likely to have had at least one biological child and are more likely to need assisted reproductive techniques to conceive.

What is known already: Childhood cancer survivors may face reproductive challenges due to the disease itself or the necessary cancer treatments. There is however a paucity of data from large population-based studies on the actual estimates of fertility, need for assisted reproduction techniques (ART) and pregnancy outcomes in young men and women childhood cancer survivors.

Study design, size, duration: We identified all individuals born in Sweden from 1958-1975 (n=2,703,888) and used linkage of national registers to follow their reproductive outcomes prospectively. Men and women with a history of childhood cancer before the age of 18 (n= 6,770) were compared with the general population without a history of childhood cancer (n=2,019,645). We assessed the occurrence of childbearing in the entire population and of trouble conceiving, use of ART and pregnancy outcomes for the first birth.

Participants/materials, setting, methods: Cancer in childhood was identified from the Cancer Register, childbearing from the Multi-generation Register and pregnancy outcomes from the Medical Birth Register. First live birth was modelled in Kaplan Meier and Cox regression, whereas risks related to the first birth were modelled with logistic regression. We performed a sibling comparison to account for unmeasured confounding by matching each individual with cancer to the nearest unaffected sibling.

Main results and the role of chance: Childhood cancer survivors were less likely to have had at least one biological child by the age of 37; hazard ratio adjusted for birth year and sex was 0.83 (95% CI: 0.80,0.86). Moreover, births were more likely to have been preceded by trouble conceiving and use of assisted reproductive techniques (ART) in both men and women surviving childhood cancer, compared to the general population. The differences were more pronounced in males than in females [males: infertility: OR, 95% CI: 1.29 (1.02,1.63); use of ART: 2.17 (1.67, 2.81); females: infertility: OR, 95% CI:1.15 (1.00, 1.32); use of ART: 1.29 (1.02, 1.61)]. Female cancer survivors had a significantly higher risk of preterm birth (<37 weeks gestation) compared to the general population (OR, 95% CI: 1.56 (1.36, 1.79), and they were also at higher risk of caesarean section. In sibling comparison, survivors of childhood cancer remained at greater risk of infertility and use of ART, and the risk of preterm birth was largely unchanged when female cancer survivors were compared to their unaffected sisters [OR, 95% CI: 1.52 (1.09, 2.10)].

Limitations, reasons for caution: Detailed information on individual cancer treatments was not available, so the effects of specific treatment regimens on fertility could not be assessed.
Wider implications of the findings: Both male and female childhood cancer survivors had poorer reproductive outcomes. These findings will help to counsel young cancer survivors and their families about the effects of cancer and cancer therapies on their future fertility and pregnancy outcomes and will help to provide individual risk estimates to guide management.
Trial registration number: Not applicable

O-038 A new strategy to assess safety of controlled ovarian stimulation protocols for oocyte vitrification as a fertility preservation technique in breast cancer patients.

M.J. Soriano[1], J. Martínez[1], R. Lopez[1], S. Herraiz[1], C. Díaz-García[1,2]

[1]IVI Foundation- IIS La Fe, Reproductive Medicine, Valencia, Spain ;
[2]IVI-RMA Global- IVI London, Reproductive Medicine, London, United Kingdom

Study question: Could controlled ovarian stimulation (COS) protocols affect the proliferative and metastatic potential of breast cancer (BC) cells?
Summary answer: Ovarian stimulation with Letrozole for oocyte vitrification might be safely used as a fertility preservation (FP) technique in hormone-dependent breast cancer women.
What is known already: BC is the most common malignancy in women at reproductive age. Treatment with high-dose chemotherapy in female patients may impose deleterious effects on the ovary. COS protocols to obtain oocytes for vitrification are frequently used within FP patients but associate a rise in supra-physiological estradiol (E2) levels. This effect could increase the proliferation of tumour cells, being probably detrimental for BC women. Nevertheless, standard COS has been recently adapted by using aromatase inhibitors such as Letrozole, leading a maximum E2 peak similar to that found in a natural cycle. Unfortunately, there is still little evidence regarding safety of such approaches.
Study design, size, duration: Experimental in vivo study. Forty 5-week old Nude-nu female mice were allocated to the following experimental groups: BC (n=10), BC and FSH stimulation (BC-FSH, n=10), BC and Letrozole stimulation (BC-LTZ, n=10), Control FSH stimulation (CT-FSH, n=5) and Control Letrozole stimulation (CT-LTZ, n=5). BC was induced in the three first groups while controls received a saline solution injection. Animals were maintained for 5 months and then sacrificed to collect tissue and blood samples for further analysis.
Participants/materials, setting, methods: One million of human MCF-7 BC cells, previously transfected with the mCherry fluorescent protein, were injected into the left renal capsule of BC, BC-FSH and BC-LTZ mice. Two days after xenograft, COS was induced by 10IU FSH or 1mg/ml Letrozole + 10IU FSH, followed by ovarian triggering with 10IU hCG at 48h. Cell proliferation was biweekly monitored by a non-invasive in vivo imagen system (IVIS) to record fluorescence signal and also assessed by Ki-67 immunostaining.
Main results and the role of chance: When tumour growth was assessed by means of total radiant efficiency signal ([p/s]/[μW/cm^2), BC and BC-LTZ mice presented a statistically significant lower expression when compared to BC-FSH group ($6.1x10^{10}$±$2.0x10^{10}$, $9.6x10^{10}$±$3.2x10^{10}$ and $1.6x10^{11}$±$4.5x10^{10}$; p<0.01 and p<0.05, respectively), five months after xenograft. Metastasis was not detected in the BC and BC-LTZ groups, nevertheless, metastatic lesions were observed in BC-FSH mice. The in vivo monitoring results by IVIS were concordant with the histological assessment of tumour lesions after sacrifice. Tumour size was slightly lower in BC group than in BC-LTZ (0.3±0.2 cm^2 vs 0.5±0.2 cm^2). However, lesions in BC-FSH group were considerably increased (1.2±0.3 cm^2, p<0.01). Cell proliferation, by Ki-67 immunostaining, was also performed in kidney samples to validate these data. Similar proliferation levels were found in the BC and BC-LTZ groups (10.3±0.9% and 11.5±0.6%). However, BC-FSH revealed a significant increase in tumour cell proliferation (28.8±1.6%, p<0.01). Lastly, mean serum E2 levels of BC and both Letrozole stimulated groups were comparable (BC: 186.9±56.0 pg/ml, BC-LTZ: 193.9±78.2 pg/ml and CT-LTZ: 119.8±50.7 pg/ml) whereas FSH-treated animals registered significantly higher E2 concentrations (BC-FSH: 431.2±56.2 pg/ml and CT-FSH: 330.9±57.0 pg/ml, p<0.05). All these results confirmed that COS with Letrozole did not induce the tumour development.
Limitations, reasons for caution: This is the first experimental study evaluating the effect of COS protocols over a human BC tumour cell line using a non-invasive in vivo system to monitor cell growth. Although the promising results, further experiments with primary human tumour cells would be required to validate the current data.
Wider implications of the findings: This study provides evidence on the safety of COS with Letrozole for BC patients. These results could be essential in reassuring current indications for FP techniques and counselling to patients and health-care professionals. Thus, patients with BC could safely underwent the gold standard FP technique, oocyte vitrification.
Trial registration number: Not applicable.

O-039 Thyroid cancer treatment and subsequent infertility diagnosis in female adolescents and young adults: a population-based cohort study

M.P. Velez[1], H. Imsirovic[2], H. Richardson[2]

[1]Queen's University, Obstetrics and Gynecology, Kingston, Canada ;
[2]Queen's University, Public Health Sciences, Kingston, Canada

Study question: Is thyroid cancer treatment associated with subsequent infertility diagnosis?
Summary answer: Thyroid cancer treatment is not associated with an increased risk of infertility diagnosis
What is known already: Thyroid cancer has undergone the most rapidly increasing incidence rate among all major cancers, most likely due to the increase of surveillance and the use of diagnostic technologies. Normal thyroid function is important to maintain normal reproduction. Previous limited literature suggests that there is little to no adverse effects of thyroid cancer treatment on gonadal function (serum gonadotrophins or sex steroids), fertility (live birth rates) or pregnancy outcomes in women treated for thyroid cancer. However, studies with larger sample sizes are needed to better elucidate the risk, if any, of different types of thyroid cancer treatment on subsequent infertility diagnosis.
Study design, size, duration: A population-based cohort study using universal health care databases in the province of Ontario, Canada. All women 15-39 years of age who received thyroid cancer treatment from 1992-2011 were identified through the Ontario Cancer Registry (N=4,926) and linked to several health care datasets. Women were followed up until December 31, 2016
Participants/materials, setting, methods: Thyroid cancer treatment was categorized as: less than total thyroidectomy-LTT, total thyroidectomy-TOT, or total thyroidectomy plus radioactive iodine therapy-TOT+RAI. Women with infertility, tubal ligation, oophorectomy, or hysterectomy previous to cancer diagnosis were excluded. Infertility diagnosis was identified using physician billed claims (ICD-9 628). Modified Poisson regression models were used to calculate the risk of infertility diagnosis adjusted for sociodemographic factors. Models were further stratified by parity at the time of cancer diagnosis (nulliparous and parous).
Main results and the role of chance: Treatment distribution was: 849 (17%) LTT, 2457 (50%) TOT, 1620 (33%) TOT + RAI. The mean age at cancer diagnosis was 31.04 years (SD=5.99). The median follow-up time for cancer survivors was 10.78 years (IQR=6.96-15.77). A total of 563 (11%) had a subsequent diagnosis of infertility. The frequency of infertility diagnosis was similar among treatments (p=0.99), with a similar mean time to infertility diagnosis (p=0.69). Mean age at infertility diagnosis was 35.26 years (SD=5.48). Compared to women with LTT, women who received TOT or TOT+RAI did not have a higher risk of subsequent infertility diagnosis (RR=0.98, 95% CI: 0.78, 1.24, p=0.86; RR=0.93, 95% CI: 0.72, 1.20, p=0.55 respectively). Parity did not modify the estimates.
Limitations, reasons for caution: The accuracy of infertility diagnosis using ICD-9 codes in administrative datasets has not been validated. Non-biologic factors that may influence the likelihood of seeking a fertility assessment may not be captured in administrative databases.
Wider implications of the findings: Thyroid cancer treatment in female adolescents and young adults is not associated with an increased risk of infertility diagnosis.
Trial registration number: not applicable

O-040 Impact of In Vitro Fertilization treatments on the risk of recurrence in fertility-sparing management of endometrial atypical hyperplasia and grade 1 adenocarcinoma

M. Vaugon[1], M. Peigné[2], E. Larouzée[1], J. Phelippeau[1], C. Gonthier[3], M. Koskas[1]

[1]Bichat Hospital, Gynecology and Obstetrics Department, Paris, France ;

[2]Jean Verdier Hospital, Assisted Reproductive Technology, Bondy, France ; [3]Pitié Salpetriere Hospital, Gynecology and Obstetrics Department, Paris, France

Study question: What is the risk of recurrence after In Vitro Fertilization (IVF) treatment for patient with endometrial atypical hyperplasia and grade 1 adenocarcinoma (AH/EC) who received conservative treatment with progestins?
Summary answer: There is no significant difference of the rate of recurrence at 24 months between patient who received IVF treatment or not.
What is known already: About 5% of endometrial cancer affect young women in reproductive age. In this particular case of AH/EC, conservative treatment, such as chlormadinone acetate, can be offered to safeguard their fertility. A quick pregnancy is recommended after progestin treatment and therefore IVF is often started. Preliminary results are encouraging: in 2013, Park's study[1] concluded that the use of fertility drugs was not associated with a higher incidence of recurrence (5-year disease free survival rate: 73% p=0,335); in 2019 Kim's study[2], the rate of recurrence was 27,3% with fertility drugs. However, they are retrospectives and not homogeneous studies.
Study design, size, duration: Multicentric prospective cohort study conducted from January 2008 to July 2019. The study is homogeneous: all the 60 patients had an AH/EC and received progestins treatment by chlormadinone acetate at least 3 months.31 patients received IVF treatment and 29 did not
Participants/materials, setting, methods: All the patients received expert advice from the National Observatory of the Gynecology and Obstetrics Department at Bichat Hospital (PREFERE center) and then enter the cohort. The choice between IVF treatment or not was made by the gynecologist and the patient, after advice from PREFERE center. Survival rates were calculated using the Kaplan-Meier method, and differences in the survival rates between groups were compared using the log-rank test.
Main results and the role of chance: The mean follow-up was 19 months in the IVF group and 11 months in no IVF group. The end of the follow-up was the time of the last news or the time of the surgery in the case of hysterectomy. The probability of 2 years recurrence was 37,7% (+/- 10,41%) in the IVF group and 55,7% (+/- 14,02%) in the no IVF group. The difference was not significant (p=0,13). We find no additional risk of recurrence according to obesity, nulliparity, PCOS, age and tumor characteristics. Twenty-three patients had a pregnancy (15 in the group IVF and 8 in the group no IVF). Among all the patients who had a pregnancy, 7 had a recurrence of the disease in the first 24 months (30%). Whereas among the 37 patients who didn't have a pregnancy, 17 patients had a recurrence in 24 months (45%). The difference was significant (p<0.01, IC 0.06-0.61). Whether the number of cycles, the E2 maximum concentration, the type of protocol chosen, the dose of gonadotrophin or the thinness of the endometrium, there were no significant differences on the risk of recurrence in the subgroup IVF.
Limitations, reasons for caution: The main inconvenient is the size of our groups but we chose to include only patient treated with chlormadinone acetate in order to have a homogenous population.
Wider implications of the findings: IVF treatment after fertility sparing management of HA/EC does not seem to increase the risk of recurrence. Therefore, it is an acceptable strategy to decrease the time to pregnancy. Overall, the rate of recurrence is high in both groups which implies a close monitoring of these patients.
Trial registration number: not applicable

O-041 Fertility preservation in male oncology patients - emergency sperm and spermatogonial stem cell retrieval

"Abstract withdrawn by the authors"

INVITED SESSION
SESSION 08: NOVEL OOCYTE AND EMBRYO BIOMARKERS
06 July 2020 Parallel 2 11:45 - 12:45

O-042 Revisited and novel fertilization biomarkers for embryo quality

I.Sfontouris[1]

[1]Eugonia, Assisted Reproduction Unit, Athens, Greece

O-043 Prediction of oocyte and embryo competence by advanced image analysis and AI

M. Zuccotti[1], **G. Fiorentino**[1], **G. Nicora**[2], **R. Sciajno**[3], **F. Cavalera**[1], **R. Bellazzi**[2], **A. Borini**[3], **S. Garagna**[1], **G. Coticchio**[3]

[1]University of Pavia, Department of Biology and Biotechnology 'Lazzaro Spallanzani', Pavia, Italy
[2]University of Pavia,
Department of Electrical- Computer and Biomedical Engineering, Pavia, Italy
[3]9.baby Fertility and Family Center, 9.baby Fertility and Family Center, Bologna, Italy

Abstract text

Our work follows that of four earlier studies. Adjuk et al. (2011) and Swann et al. (2012) first explored the potential of particle image velocimetry (PIV) (an optical method to measure fluid and particles dynamics) for the study of cytoplasmic dynamics occurring during mouse fertilisation. Bui et al. (2017) and Cavalera et al. (2018), by complementing the detection of cytoplasmic flows with artificial neural networks (ANN), further perfected this imaging approach, succeeding in the prediction of the developmental competence of mouse oocytes. After the presentation of these results, we will describe our latest work on human preimplantation embryos.

The ANN approach was undertaken to assess retrospectively the ability of human embryos to develop to the blastocyst stage. The analysis focused on 113 embryos generated in 32 IVF cycles, carried out between October 2015 and May 2018. Female age was 36.3±4.9 years. To minimize possible patient-based bias, cycles were recruited ensuring to have in the same cohort both embryos able to develop to the blastocyst stage and arresting at earlier stages. Embryos were subject to time-lapse assessment to monitor development and perform trophectoderm biopsy for preimplantation genetic testing of aneuploidy. Fertilisation was achieved by ICSI. Time-lapse monitoring started immediately after ICSI, with a 15 min interval between consecutive observations. Of 230 embryos analysed, 112 reached the blastocyst stage (BL-group) and 118 arrested sometime after the 2-cell stage (NoBL-group). ANN analysis was performed, at this stage, only during the first two cell divisions (44 hr, 175 frames).

Based on morphological criteria, embryos were first blindly classified by an expert operator as belonging to the BL or NoBL group, attaining a 75.4% accuracy, 76.5% sensitivity and 74.3% specificity. Then, the time-lapse images corresponding to the first 175 frames (44 hr) were anlaysed with PIV to extract their cytoplasmic movement profiles, and subsequently with the AI models (k-NN and LSTM-NN). The results showed a classification accuracy comparable to that reported by the operator (75.4%). Next, the results of the operator were integrated to those obtained with the AI models, and this led to a further classificaton attaining a 82.6% accuracy, 79.4% sensitivity and 85.7% specificity. Taken as a whole, these data indicate the possibility of predicting blastocyst development as early as the 4-cell stage; also, these results highlight the strenght of combining the huge experience owned by an expert clinical embryologist with that obtained thanks to AI tools.

The study represents a proof-of-principle for the chosen embryo assessment approach. Further observations are required to trenghten these data and to assess the impact of possible confounding variables such as patient typologies, culture conditions and time-lapse equipment.

To the best of our knowledge this study represents the first attempt to classify very early human preimplantation embryos using AI. Further refinement of the approach is expected to impact embryo assessment ability and improve efficiency in assisted reproduction treatments.

INVITED SESSION
SESSION 09: DATA REPORTING SESSION: THE EUROPEAN PERSPECTIVE (EIM AND PGT)
06 July 2020 Parallel 3 11:45 - 12:45

O-044 Assisted Reproductive Technology (ART) in Europe 2016 and development of a strategy of vigilance – Preliminary results generated from European registers by the ESHRE EIM Consortium

C. Wyns[1], C. Bergh[2], C. Calhaz-Jorge[3], Ch. De Geyter[4], M.S. Kupka[5], T. Motrenko[6], A. Rugescu[7], J. Smeenk[8], A Tandler-Schneider[9], S. Vidakovic[10], V. Goossens[11]

[1]Cliniques universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium
[2]Dept of Obstetrics and Gynecology, Inst of Clinical Sciences, Göteborg University, Göteborg, Sweden
[3]Faculdade de Medicina da Universidade de Lisboa, Portugal
[4]Reproductive Medicine and Gynecological Endocrinology (RME), University Hospital, University of Basel, Switzerland
[5]Fertility Center - Gynaekologicum, Hamburg, Germany
[6]Human Reproduction Center Budva, Montenegro
[7]National Transplant Agency, Romania
[8]Elisabeth Twee Steden Ziekenhuis, Tilburg, the Netherlands
[9]Fertility Center Berlin, Berlin, Germany
[10]Institute of Obstetrics and Gynecology, Clinical Center Serbia «GAK», Serbia
[11]ESHRE Central Office, Meerstraat 60, Grimbergen, Belgium

Study question: What are the reported data on ART cycles, IUI and fertility preservation (FP) interventions in 2017 as compared to previous years, as well as main trends over the years?
Summary answer: The 21st report shows a progressive increase in reported treatment cycle numbers in Europe, a slight decrease in the number of transfers (IVF + ICSI) with more than one embryo, stable multiple delivery rates (DRs), unchanged outcomes for IUI cycles and higher pregnancy rates (PRs) and DRs after FER compared to fresh IVF and ICSI cycles.
What is known already: Since 1997, ART aggregated data generated by national registries and professional organisations have been collected, analysed by the European IVF-monitoring Consortium (EIM) and reported in 20 manuscripts published in *Human Reproduction* and *Human Reproduction Open*.
Study design, size, duration: Yearly collection of European medically assisted reproduction and FP data by EIM for ESHRE; data on treatments performed between January 1 and December 31 2017 in 30 European countries were provided by either National Registries or registries based on professional organisations.
Participants/materials, setting, methods: 1229 clinics offering ART services in 30 countries reported a total of 840 138 treatment cycles, involving 147 934 with IVF, 351 588 with ICSI, 238 665 with frozen embryo replacement (FER), 33 721 with preimplantation genetic testing (PGT), 62 811 with egg donation (ED), 369 with IVM of oocytes and 5050 with frozen oocyte replacement (FOR). Data on IUI using husband/partner's semen (IUI-H) and donor semen (IUI-D) were reported from 1170 institutions, including 141 647 treatments with IUI-H in 24 countries and 48 918 treatments with IUI-D in 20 countries. A total of 18 540 FP interventions from 11 countries including oocytes, semen, as well as ovarian and testicular tissue in pre-and postpubertal patients were reported.
Main results and the role of chance: 1229 IVF clinics (94.5% of registered clinics in participating countries) and 1170 IUI providers reported their data. In the 30 reporting countries, after IVF the clinical pregnancy rates (PRs) per aspiration and per transfer were 29.5% and 39.4%, respectively (versus 28.5% and 34.6% in 2016). Corresponding rates after ICSI were 27.2% en 39.8% (versus 26.2% and 33.2% in 2016). After FER with own embryos the PR per thawing is still on the rise, from 30.9% in 2016 to 31.9% in 2017. After ED the PR per fresh embryo transfer was 49.1% (49.4% in 2016) and per FOR 43.1% (43.6% in 2016).

In IVF and ICSI together, the trend towards the transfer of fewer embryos continues with the transfer of 1, 2, 3 and ≥4 embryos in 45.4%, 49.8%, 4.5% and 0.2% of all treatments, respectively (versus 41.5%, 51.9%, 6.2% and 0.4% in 2016). This resulted in a proportion of singleton, twin and triplet DRs of 85.4%, 14.3% and 0.3%, respectively (versus 84.8%, 14.9% and 0.3% in 2016). Treatments with FER resulted in twin and triplet DRs of 11% and 0.2%, respectively (versus 11.9% and 0.2% in 2016).

After IUI, the DRs remained similar at 8.7% after IUI-H (8.9% in 2016) and at 12.2% after IUI-D (12.4% in 2016). Twin and triplet DRs after IUI-H were 8.5% and 0.3%, respectively (in 2016: 8.8% and 0.3%) and 7.0% and 0.2% after IUI-D (in 2016: 7.7% and 0.4%). The majority of FP interventions included the cryopreservation of ejaculated sperm (n=10837 from 11 countries) and of oocytes (n=6551 from 11 countries).
Limitations, reasons for caution: As the methods of data collection and levels of completeness of reported data vary among European countries, the results should be interpreted with caution. Due to the COVID-19 pandemic, less countries than usual have sent in data by the abstract deadline.

O-045 Data from the ESHRE PGT Consortium – year 2018

A. Van Montfoort[1], M. De Rycke[2], F. Carvalho[3], C. Rubio[4], F. Bronet[5], F. Spinella[6], V. Goossens[7]

[1]Maastricht University Medical Center, Dept. of Ob/Gyn, Maastricht, The Netherlands
[2]UZ Brussels, Centre for Medical Genetics, Brussels, Belgium
[3]University of Porto Faculty of Medicine, Genetics Faculty of Medicine, Porto, Portugal
[4]Igenomix, R&D Department, Valencia, Spain
[5]IVI, IVF and PGD lab, Madrid, Spain
[6]Genoma, Molecular Genetics Laboratories, Rome, Italy
[7]ESHRE, Central Office, Grimbergen, Belgium

Study question: Which trends are shown in data collection XXI of the European Society of Human Reproduction and Embryology (ESHRE) PGT Consortium compared with previous years?
Summary answer: Data collection XXI, year 2018, represents valuable data on PGT activity in (mainly) Europe and reports on the main trends observed which are the continuous increase of trophectoderm biopsy and further expansion of comprehensive testing technology in PGT-SR and PGT-A.
What is known already: The ESHRE PGT Consortium was set up in 1997 and from that time has been collecting data on PGT and PGT-A. The PGT database comprises the world's largest collection of PGT / PGT-A data providing a valuable resource for data mining and for following trends in PGT practice. So far, up to the year 2015, data collections were carried out in a retrospective data way, from 2016 onwards a prospective data collection was in place.
Study design, size, duration: As the nature of PGT/ PGT-A treatments has changed significantly over the last years and IVF cycle management and genetic analysis techniques are getting more complex, ESHRE uses an online data collection system in which data are collected prospectively from oocyte retrieval to analysis, embryo transfer and pregnancy / live birth. Data are collected cycle by cycle on a voluntary basis.
Participants/materials, settings, method: For the 2018 data, individual centres (39) from 21 countries directly entered the data into the PGT database through software developed by ESHRE. Data were analysed at ESHRE headquarters and include all aspects of PGT/PGT-A cycles.
Main results and the role of chance: The Consortium has analysed the PGT analyses (n=28231) performed in 2018. The indications for PGT included inherited chromosomal abnormalities (n=553 analyses), monogenic disorders (n=1229 analyses), aneuploidy testing for infertility (n=868 analyses), HLA typing (n=12 analyses) and PGT for mitochondrial disorders (n=3 analyses). In addition, 701 clinical pregnancies and 411 deliveries have been analysed in detail. The methods used for biopsy were polar body (1.1%), cleavage stage biopsy (31.4%) and blastocyst biopsy (67.5%; comparable with data from 2017). The methodology used for diagnosis is also evolving, with data set XXI showing around 10% of FISH, 37% of PCR and 53% of WGA. Within WGA 79% of the analyses were done using NGS, 10% using array-CGH and in 8.5% cases SNP arrays were used. The overall pregnancy rate is about 21.5%. The baby data show that it is difficult for most centres to have a detailed follow-up.
Limitations, reasons for caution: The findings apply to the 38 participating centres and may not represent worldwide trends in PGT. Data were collected prospectively, but details of the follow-up on PGT pregnancies and babies born were limited.
Wider implications of the findings: The ESHRE PGD Consortium continues its activities as an important forum for PGT practitioners to share data and exchange experiences. The information extracted from the data collections helps to monitor quality issues in PGT and survey the introduction and effectiveness of new PGT technologies and methods

INVITED SESSION

SESSION 10: UPDATED TERMINOLOGY FOR EARLY PREGNANCY ASSESSMENT

06 July 2020 Parallel 4 11:45 - 12:45

O-046 Can you be sure that the pregnancy is Intrauterine?

D.Jurkovic[1]

[1]*University College Hospital, Department of OB/GYN, United Kingdom*

O-047 The ectopic pregnancies you cannot see with a laparoscope

E. Kirk[1]

[1]*Royal Free Hospital London, Early pregnancy and emergency Gynaecology Unit, United Kingdom*

INVITED SESSION

SESSION 11: PATIENT PRIORITIES

06 July 2020 Parallel 5 11:45 - 12:55

O-048 Genetic ancestry testing and the end of donor anonymity

D.Kennett[1]

[1]*University College London, Department of Genetics- Evolution and Environment, London, United Kingdom*

Abstract text

Over 30 million people around the world have now taken a direct-to-consumer genetic test with companies such as AncestryDNA and 23andMe. These tests can provide information about an individual's genetic ancestry and health but they also allow the user to opt in to receive matches with genetic relatives. As a result, donor-conceived individuals can now take a DNA test to search for their DNA relatives and identify their genetic parent. Identities can be inferred through networks of genetic cousins even if the donor is not in the database. The chances of success are increasing all the time and sometimes people are matched at the outset with a half-sibling or genetic parent. Donor origins can also be discovered unexpectedly through genetic testing if the child has not been told that they were donor conceived. The massive growth of the consumer DNA databases in the last few years has important implications for everyone working in the fertility industry and for parents who are considering using egg or sperm donation to start or complete their family. DNA testing has effectively ended anonymity for all donors regardless of the legislation in place at the time. How do we deal with the challenges of this new reality? Whose responsibility is it to provide support and counselling for those affected? How should the fertility sector adapt?

Trial registration number:
Study funding:
Funding source:

O-049 Infertility - the political treatment

A.Fincham[1]

[1]*Fertility Europe VZW, Evere, Belgium*

INVITED SESSION

SESSION 12: ASRM EXCHANGE SESSION - CONTROVERSIES IN ART

06 July 2020 Parallel 2 14:00 - 15:00

O-051 Endometrial evaluation for all?

C.Coutifaris[1],

[1]*University of Pennsylvania Medical Centre, Division of Reproductive Endocrinology and Infertility, U.S.A*

O-052 Is the male gamete relevant in ART?

P.N. Schlegel[1]

[1]*Cornell Institute for Reproductive Med., Department of Urology- Starr 900, New York- NY, U.S.A.*

Abstract text

The critical role of female factors, especially female age, on the outcomes of assisted reproductive technologies is well recognized. Fewer data have been generated regarding the role of sperm or male factors on IVF results. Initially, sperm factors were recognized to adversely affect the fertilization step of in vitro fertilization, with variable interpretation of the reproductive competency of the embryo derived using sperm from men with abnormal semen parameters. With the advent of ICSI for management of male factor, the risk of fertilization failure due to sperm abnormalities has been largely overcome. Several male-related factors, including paternal age, have been identified that affect the outcome of IVF. One pathway for male factors to adversely affect IVF results includes sperm DNA integrity. Abnormal sperm DNA fragmentation is rarely seen in naturally fertile men, but can be present even in the presence of normal semen parameters. Sperm DNA damage can be evaluated by a variety of assays, and appears to develop during sperm transport (i.e., after leaving the testis) for the majority of men with abnormal sperm DNA fragmentation index (DFI.) It may be associated with ejaculatory dysfunction (spinal cord injury), partial obstruction (post-vasectomy), medications that effect ejaculatory efficiency (SSRI's), prolonged abstinence, advanced male age, or without identifiable cause. Abnormal sperm DFI is associated with a marked increase in the risk of spontaneous Ab, as well as decreased chance of pregnancy with IVF/ICSI. The evaluation and management of abnormal ejaculated sperm DFI will be reviewed as well as the existent data on the effect of DNA fragmentation on assisted reproductive technology results.

INVITED SESSION

SESSION 13: CHALLENGING SCENARIOS IN IVF PATIENTS

06 July 2020 Parallel 3 14:00 - 15:00

O-053 The real place of IVF in PCOS

G.Lainas[1]

[1]*Eugonia, IVF Unit, Athens, Greece*

O-054 Low responders: From natural cycles to dual stimulation?

F. Ubaldi[1], **A. Vaiarelli**[2], **D. Cimadomo**[2], **L. Rienzi**[2]

[1]*Reproductive Medicine GENERA, Centre for Reproductive Medicine, Roma, Italy*
[2]*Genera, Centre for reproductive medicine, Rome, Italy*

Abstract text

"Low responders: From natural cycles to dual stimulation?"

Filippo Maria Ubaldi, MD, PhD; clinical director of GENERA, centers for reproductive medicine, Italy

The lack of a consensus on the definition of "poor responders" (POR) in the past made it difficult to compare the proposed managements among each other. A systematic review highlighted 41 different definitions of POR out of 47 randomized control trials (RCTs). In this scenario, the Bologna criteria were the first concrete attempt to define POR grounded on an evidence-based approach. Noticeably, the predicted cumulative live birth rate per IVF cycle in patients fulfilling these criteria is not higher than 7-8% according to the existing evidence in the literature.

Recently, a panel of clinicians gathered together in the so-called POSEIDON group and suggested a novel more detailed stratification of women with a low response to controlled ovarian stimulation (COS). This group introduced a new concept in IVF: the main aim of COS is to obtain at least one embryo with a high chance to result in a live birth, namely euploid blastocyst(s). Several data exist in the literature supporting that the chance of a blastocyst to be euploid is independent of COS, its regimen, the dose of gonadotrophins used, and the number of oocytes/embryos in each cohort. Therefore, the careful choice and tailoring of the strategy that better suits each patients is critical.

The obvious and mostly-used clinical strategy for women with low AFC and/or AMH was to increase the daily dose of gonadotrophins but unfortunately without success. In fact, gonadotrophins can only support each follicular wave in its growth, but they cannot produce follicles de novo. Natural cycle has been also adopted across the years. However, it is mainly effective in patients with an expected number of oocytes retrievable lower than 2-3. The adoption of COS might be helpful, as also suggested by the recent ESHRE guidelines.

Another strategy has been outlined across the years to treat time-sensitive (oncologic and advanced maternal age) and POR patients, which is known as DuoStim (follicular and luteal phase stimulation in the same ovarian cycle). This strategy is supported from the evidence that 2-3 waves of follicular growth might arise during the ovarian cycle in women, 1-2 of which in the luteal phase. Although the ESHRE labeled this strategy still as "for research only", several evidences have been produced along the last 5 years from our group, as well as from many other groups:

- we set-up the DuoStim protocol in a pilot study,
- we confirmed the absence of differences in terms of fertilization, blastulation and euploidy rates between the cohorts of oocytes collected from the luteal and the paired-follicular phase,
- we reported the reproducibility of the DuoStim protocol among all the GENERA centers
- we reported similar clinical, obstetrical and neonatal outcomes among euploid blastocysts obtained after follicular and luteal phase stimulation through a non-selection study design
- we suggested DuoStim as a strategy to prevent drop-out in patients fulfilling the Bologna criteria, while increasing their chance of having a live birth per intention to treat from 8 to 15%
- we conducted a systematic review that highlighted the reproducibility of our data also in other clinics adopting slightly different protocols
- from a basic research perspective, we collaborate with Universities to keep building evidence in favor of DuoStim safety

Clearly RCTs and cost-effective analyses are still needed but, when indicated and depending on the setting of each IVF clinic, DuoStim might be considered a valuable strategy to prevent patients' drop-out while increasing their chance to identify at least one euploid blastocyst in the shortest possible time.

INVITED SESSION

SESSION 14: THE WAY FORWARD FOR FERTILITY PRESERVATION

06 July 2020 Parallel 4 14:00 - 15:00

O-055 Cancer during pregnancy: Outcomes for mother and child

F. Amant[1]

[1]*Netherlands Cancer Institute, The Netherlands*

O-056 Fertility preservation for children: Specificities and counselling for patients and parents

G. Quinn[1]

[1]*New York Univeristy, OB-GYN, New York, U.S.A.*

Abstract text

The use of fertility preservation in children receiving gonadotoxic therapies is often ethically challenging. Presently most countries have some type of guidelines suggesting all patients receiving a cancer or other life-threatening diagnosis, receive information on how that may impact their future fertility and be offered referrals to specialist to discuss preservation. Not all types of preservation are available in certain countries and in some cases, finances become a rate limiting factor as well as potential delay of treatment. Parents may feel uncertain about their role in making future decisions about their children's future fertility and health care clinicians may also be concerned that engaging in discussions and consultations may delay the lifesaving treatment needed. There are also psycho-social issues involved in the decision process both at the time of the diagnosis as well as in the future. There has been great controversy over the use of posthumous assisted reproduction, particularly the use of gametes stored by a minor and later used by a parent to create a grandchild. There is also the unique circumstance of the transgender/non-binary child and their family who may be considering puberty- blockers or gender affirming hormones. This presentation will explore the ethical and psycho-social issues and boundaries of the use of fertility preservation in children (minors) and explore the current related literature. The objectives of this presentation are to:

1. Identify current standard and experimental fertility preservation options for minors
2. Discuss ethical challenges faced by parents and healthcare clinicians
3. Explore current cases on decision-making and use of fertility preservation in minors
4. Compare and contract the ethics of FP on very young children (aged 0-7) compared to middle aged children (8-14) and older children (15-18)
5. Examine the issue of fertility preservation in transgender/non-binary children

SELECTED ORAL COMMUNICATIONS

SESSION 15: IN THE NAME OF THE FATHER

06 July 2020 Parallel 5 14:00 - 15:00

O-057 Septum resection versus expectant management in women with a septate uterus: a randomised controlled trial (NTR 1676)

J. Rikken[1], **C. Kowalik**[1], **M.H. Emanuel**[2], **M. Bongers**[3], **T. Spinder**[4], **F.W. Jansen**[5], **A. Mulders**[6], **R. Padmehr**[7], **J. Clark**[8], **H. Van Vliet**[9], **M. Stephenson**[10], **F. Van Veen**[11], **B.W. Mol**[12], **M. Van Wely**[11], **M. Goddijn**[11]

[1]*Academisch Medisch Centrum, Center for Reproductive Medicine, Amsterdam, The Netherlands ;*
[2]*University Medical Center Utrecht, Obstetrics & Gynaecology, Utrecht, The Netherlands ;*
[3]*Maxima Medical Center, Obstetrics & Gynaecology, Veldhoven, The Netherlands ;*
[4]*MC Leeuwarden, Obstetrics & Gynaecology, Leeuwarden, The Netherlands ;*
[5]*LUMC, Obstetrics & Gynaecology, Leiden, The Netherlands ;*
[6]*Erasmus MC, Obstetrics & Gynaecology, Rotterdamthe, The Netherlands ;*
[7]*Avicenna Research Institute, Obstetrics & Gynaecology, Teheran, Iran ;*
[8]*Birmingham women's and children's hospital, Obstetrics & Gynaecology, Birmingham, United Kingdom ;*
[9]*Catharina hospital, Obstetrics & Gynaecology, Eindhoven, The Netherlands ;*
[10]*University of Illinois. Chicago, Obstetrics & Gynaecology, Chicago, The Netherlands ;*
[11]*AMC, Obstetrics & Gynaecology, Amsterdam, The Netherlands ;*
[12]*Monash Medical Center, Obstetrics & Gynaecology, Clayton, Australia*

Study question: Does hysteroscopic septum resection improve reproductive outcomes in women with a septate uterus?

Summary answer: In our study, hysteroscopic septum resection did not improve reproductive outcomes in women with a septate uterus.
What is known already: The septate uterus is the most common uterine anomaly with an estimated prevalence of 0.2-2.3% in women of reproductive age. Women with a septate uterus are at increased risk for subfertility, pregnancy loss and preterm birth. Hysteroscopic septum resection has been applied to improve reproductive outcome in these women, but the evidence on the effectiveness of this procedure is scarce and of low quality. We performed a randomised controlled trial to establish whether septum resection improves reproductive outcome in women with a septate uterus.
Study design, size, duration: We did an international open-label randomised controlled trial in 10 centres in the Netherlands, United Kingdom, United States of America and Iran. The trial is registered with the Netherlands Trial Register as NTR 1676.
Participants/materials, setting, methods: Women with a septate uterus and a history of subfertility, pregnancy loss or preterm birth were randomly allocated to septum resection or expectant management. The primary outcome was live birth within 12 months after randomization. Secondary outcomes were clinical pregnancy, ongoing pregnancy, pregnancy loss, preterm birth and complications following hysteroscopic septum resection. We analysed the data on intention to treat basis. We estimated differences as relative risks (RR) with 95% confidence intervals (CI).
Main results and the role of chance: Between October 2010 and October 2018 we randomly assigned 80 women (40 to septum resection and 40 to expectant management). Live birth occurred in 12 women who underwent septum resection (32.4%) and in 14 women who had expectant management (37.8%) ((RR 0.89 (95% CI 0.56-1.41). Clinical pregnancy occurred in 22 women who underwent septum resection (59.5%) and in 19 women who had expectant management (51.4%) (RR 1.18 (95% CI 0.75-1.85); pregnancy loss occurred in 10 women who underwent septum resection (43.5% of women who had a conception) and in 5 women who had expectant management (26.3% of women who had a conception) (RR 1.56 (95% CI 0.70-3.47); ongoing pregnancy occurred in 13 women who underwent septum resection (35.1%) and in 14 women who had expectant management (37.8%) (RR 0.92 (95% CI 0.58-1.48); preterm birth occurred in 5 women who underwent septum resection (38.5% of women who had an ongoing pregnancy) and in 4 women who had expectant management (28.6% of women who had an ongoing pregnancy) (RR 1.33 (95% CI 0.48-3.70). In women who underwent septum resection, one perforation of the uterus occurred during surgery. The procedure was stopped immediately and the septum was removed 4 weeks later without complications.
Limitations, reasons for caution: Our major limitation is the limited sample size.
Wider implications of the findings: Our results suggest that septum resection does not result in better pregnancy outcomes. This procedure is not without risks and should not be offered to women without proper counselling. We believe an expectant management is the preferred primary treatment in women with a septate uterus.
Trial registration number: NTR 1676

O-058 Hysteroscopic Fundus Endometrial Incision (FEI) in oocyte recipients before embryo transfer (ET) during In Vitro Fertilization

R. Najdecki[1], E. Papanikolaou[1], T. Chartomatsidou[1], F. Pakaki[1], S. Stamataki[1], P. Tatsi[1], E. Timotheou[1], F. Chouliara[1], E. Bampas[1], S. Bouchlariotou[1], G. Michos[2], A. Athanasiadis[2]

[1]Assisting Nature, IVF Unit, Thessaloniki, Greece ;
[2]Aristotle University, 3rd Department Ob Gyn, Thessaloniki, Greece

Study question: Does Hysteroscopic Fundus Endometrial Incision (FEI) in oocyte recipients before embryo transfer increase pregnancy and live birth rates?
Summary answer: Oocyte recipients who underwent FEI before ET showed statistically higher live births rates.
What is known already: Controversial results are published regarding the necessity of hysteroscopy plus uterine scratching or not before ET. For oocyte recipients, who might have previously undergone several failed IVF procedures, the importance of correct evaluation of uterine cavity is of paramount importance since there are limited psychological resources to sustain potential failure.
Study design, size, duration: Between 2014-2019, 332 egg recipients were assigned prospectively in a ratio 1:2 to undergo hysteroscopy (n=114, **Hysteroscopy group**) or not (n=218,**Non-Hysteroscopy group**). These women, who performed pre-operative hysteroscopic evaluation of the uterine cavity, also underwent fundus endometrial incision irrespectively of the presence of arcuate uterus (U2a) or not (U0) with endoscopic scissor. The relation of the FEI to pregnancy outcome was investigated.
Participants/materials, setting, methods: The age of the women included in the study ranged from 35-50 years old. The mean years of infertility was 4,6. Women received treatment with donor oocytes and the mean blastulation rate was 59%. They underwent embryo transfer with two blastocysts except from 10 cases which preferred single blastocyst transfer to avoid twins.
Main results and the role of chance: Among 114 women of the Hysteroscopy group(Storz Bettocchi 5mm) prior to embryo transfer,33 were diagnosed with U2a (partial septate, arcuate uterus), 10 with U2b (complete septate uterus) and 6 women with UIa (T shape uterus). Both women with uterine abnormality (n=49) plus the ones with normal cavity (n=65) underwent Fundus Endometrial Incision with Wolf endoscopic scissor. The rest 218 recipients did not undergo hysteroscopy prior to ET. Initial positive human Chorionic Gonadotropin (hCG) test was 73.7% in the hysteroscopy group and 57,34% in the non-hysteroscopy group. Live Birth rate was statistically significantly (p=0,015) higher in the hysteroscopy group in a rate of 56,14% (n=64/114) compared to the live birth rate of 42,2% (n=92/218) in the non-hysteroscopy group.
Limitations, reasons for caution: The sample size of the participants should be expanded in order to obtain more solid evidence of the impact of FEI in pregnancy outcomes. Moreover, the time interval between the hysteroscopy and the ET should be taken into further consideration.
Wider implications of the findings: The current findings indicate that in this particular subgroup of patients- like oocyte recipients- hysteroscopy plus FEI might increase delivery rates. Apart from the obvious benefit of recognizing obscured anomalies, requiring surgical correction, it appears that even fundus incision might improve uterine receptivity and thus pregnancy outcome.
Trial registration number: none

O-059 Improvement of symptoms after hysteroscopic isthmoplasty in women with abnormal uterine bleeding and expected pregnancy: a prospective study

D. Tran[1], T. Nguyen[2], D. Do[3], H. Nguyen[4], P. Thuong[2]

[1]Ha Noi Obstetric and Gynecology hospital, Department of high risk pregnancy, Ha Noi, Vietnam ;
[2]Ha Noi Obstetric and Gynecology hospital, Delivery department, Ha Noi, Vietnam ;
[3]Ha Noi Obstetric and Gynecology hospital, Gynecology department, Ha Noi, Vietnam ;
[4]Ha Noi Obstetric and Gynecology hospital, Assisted Reproductive Center, Ha Noi, Vietnam

Study question: To determine the effectiveness and safety of hysteroscopic repair of isthmocele to treat postmenstrual spotting and the rate of natural pregnancy after surgery
Summary answer: Our data supports that hysteroscopic isthmoplasty is safe and effective in treating isthmocele-related abnormal uterine bleeding
What is known already: A cesarean scar defect, also called as an isthmocele, is one of the consequences of cesarean section that may cause secondary infertility and postmenstrual spotting.
Study design, size, duration: 23 patients with abnormal uterine bleeding and expected pregnancy who had an isthmocele with a residual myometrium of ≥2.5mm, measured during transvaginal untrasound were performed hysteroscopic repair of isthmocele
Participants/materials, setting, methods: 23 patients with abnormal uterine bleeding and expected pregnancy who had an isthmocele with a residual myometrium of ≥2.5mm,were performed hysteroscopic repair of isthmocele using a step-wise approach: identification of relevant anatomy; resection of the cephalad edge of fibrosis; resection of the caudad edge of fibrosis; and ablation of the isthmocele base. Complications of surgery were observed and the symtoms of postmentstrual spotting and prevalence of natural pregnancy were monitored for 6 months after surgery.

Main results and the role of chance: No complications of surgery as bleeding, uterine perforation, infection was noticed. 11 (47.8%) patients had resolution of their symptoms. In the remaining cases, 7 (30.4%) patients had an improvement of symtoms with decreasing duration of postmentstrual spotting from 8.1 ±1.7 days to 4.3 ± 0.8 days ($P < 0.05$), whereas 5 (21.8%) patients did not obtain any relief. 7 (30.4%) patients had natural pregnancy after surgery 6 months.
Limitations, reasons for caution: The sample size is not enough large
Wider implications of the findings: Hysteroscopic isthmoplasty should be used to treat isthmocele-related abnormal uterine bleeding
Trial registration number: Not applicable

O-060 Conception and Reproductive Outcomes in Asherman Syndrome after hysteroscopic adhesiolysis

S. Baradwan[1], D. Alharbi[2], M. Bashir[3], A. Saleh[3], D. Al-Jaroudi[4]

[1]HealthPlus Fertility and Women's Health Center- Jeddah- Saudi Arabia., Department of Obstetrics and Gynecology, jeddah, Saudi Arabia ;
[2]King Fahad Medical City- Riyadh- Saudi Arabia, Department of Obstetrics and Gynecology., Riyadh, Saudi Arabia ;
[3]King Fahad Medical City- Riyadh- Saudi Arabia, Research Services Administration-Department of Biostatistics- Research Center., Riyadh, Saudi Arabia ;
[4]King Fahad Medical City- Riyadh- Saudi Arabia, Reproductive Endocrine and Infertility Medicine Department., Riyadh, Saudi Arabia

Study question: Dose the reproductive methods could influence pregnancy outcome in women with Asherman's syndrome ?
Summary answer: The spontaneous cumulative conception rates following hysteroscopic adhesiolysis were higher than those for the IVF-ICSI, whereas the live birth and miscarriage rates were similar.
What is known already: Hysteroscopic adhesiolysis anatomically restores the uterine cavity in cases of Asherman's syndrome. however, the extent of endometrial fibrosis could lead to implantation failure and miscarriages. The main purpose of treating infertile women with Asherman's syndrome is improve both the conception rate and the live birth rate. Few studies have evaluated the reproductive outcome after hysteroscopic adhesiolysis.
Study design, size, duration: A retrospective cohort study that included 41 women diagnosed with Asherman syndorme who attended Women's Specialized Hospital, King Fahad Medical City, from December 2010 to December 2016 and presented with a history of infertility or recurrent pregnancy loss. Patients were followed up for 2 years to monitor pregnancy. Details of reproductive methods whether with spontaneous conception or assisted reproductive technology were recorded. The main outcome measure was identification of reproductive methods and pregnancy outcome.
Participants/materials, setting, methods: all women diagnosed with Asherman syndorme who attended Women's Specialized Hospital, King Fahad Medical City, presented with a history of infertility or recurrent pregnancy loss. Patients were followed up for 2 years to monitor pregnancy. Details of reproductive methods whether with spontaneous conception or assisted reproductive technology were recorded. The main outcome measure was identification of reproductive methods and positive pregnancy tests that might have ended in miscarriage or ectopic, live birth, or no pregnancy.
Main results and the role of chance: The overall conception rate was (53.6%, n=22) after hysteroscopic adhesiolysis. The live birth rate was (34.2%, n=14) the miscarriage rate was (14.6%, n=6) and the ectopic pregnancy rate was (4.9%, n=2). The conception rate was significantly (P=0.037) higher in spontaneous conception compared with IVF- ICSI, 80% (12 out of 15) compared to 44.4% (8 out of 18), respectively. The live birth and miscarriage rate were reported by 53.3% (8 out of 15), 20% (3 out of 15) of the spontaneous conception group compared to 27.8% (5 out of 18), 16.7% (3 out of 18) of the IVF-ICSI group, respectively. This was statistically not significant.
Limitations, reasons for caution: Among the limitations of this study is the small size of the sample, as it was limited to one Hospital, which could affect the generalizability. Another limitation is the study's retrospective nature, second look hysteroscopy was not performed, and the menstrual pattern after the procedure was not documented.
Wider implications of the findings: The spontaneous cumulative conception rates following hysteroscopic adhesiolysis were higher than those for the IVF-ICSI, whereas the live birth and miscarriage rates were similar.
Trial registration number: 19-024

INVITED SESSION
SESSION 16: BREAKING NEWS IN CURRENT PRACTICE

06 July 2020 Parallel 6 14:00 - 15:00

O-061 Fresh or frozen? - First results of e-freeze trial from UK

A. Maheshwari[1]

[1]University of Aberdeen, United Kingdom

O-062 Recommendations for good practice for the use of time-lapse technology

D. Montjean[1], S. Apter[2], T. Ebner[3], T. Freour[4], Y. Guns[5], B. Kovacic[6], N. Le Clef[7], M. Marques[8], M. Meseguer[9], I. Sfontouris[10], R. Sturmey[11], G. Coticchio[12]

[1]Hopital Saint-Joseph, Service de médecine et biologie de la reproduction, Marseille, France
[2]Livio, Livio, Stockholm, Sweden
[3]Kepler Universitätsklinikum, Department of Gynecology- Obstetrics- and Gynecological Endocrinology, Linz, Austria
[4]CHU de Nantes, Medecine de la Reproduction, Nantes, France
[5]UZ Brussel, Center for Reproductive Medicine, Brussels, Belgium
[6]Univerzitetni klinicni center Maribor-, Department of Reproductive Medicine and Gynecologic Endocrinology-, Maribor, Slovenia
[7]European Society of Human Reproduction and Embryology, Guidelines, Grimbergen, Belgium
[8]CEMEARE, cemeare, Lisbon, Portugal
[9]Instituto Valenciano de Infertilidad, IVF Laboratory, Valencia, Spain
[10]Eugonia, Assisted Reproduction Unit, Athens, Greece
[11]Hull York Medical School- University of Hull, Centre for Atherothrombosis and Metabolic Disease-, Hull, United Kingdom
[12]9.baby Family and Fertility Center, 9.baby Family and Fertility Center, Bologna, Italy

Abstract text

Traditional embryo morphology assessment is performed at static time points and implies interruption of embryo culture conditions. In a TLT incubator, images of embryo development are recorded at regular time intervals, which facilitates embryo monitoring. Indeed, it allows embryologists to assess embryo development thoroughly in a dynamic fashion without removing them from the incubator and thus maintaining constant culture conditions. Although TLT has been widely implemented since its release in 2010, there was no recommendation on how to introduce this technology in an IVF laboratory and no review of other aspects of its use. To address this need, a working group was constituted. It included 11 members of different nationalities with internationally recognized experience in clinical embryology and basic science embryology. The stakeholders of this project have reviewed the literature and collected published surveys and manufacturer information up to January 2019. Once a consensus was found on the content of the recommendation manuscript, a draft was released on ESHRE website for review by ESHRE members. The paper was published in HRopen (https://academic.oup.com/hropen/article/2020/2/hoaa008/5809428) and the recommendations are available on ESHRE website (https://www.eshre.eu/Guidelines-and-Legal/Guidelines/TLT). The working group listed 11 recommendations on what to do before introducing TLT in an IVF laboratory. These statements include an assessment of the pros and cons of acquiring a TLT system, selection of relevant morphokinetic parameters, selection of an appropriate TLT system with technical and customer support, development of an internal checklist and education of staff. This paper also addresses more general aspects of TLT introduction in IVF laboratory such as the potential benefit of TLT especially regarding embryo quality assessment and the identification of

parameters with biological/clinical outcomes. This document discusses in what extent TLT helps in embryo selection/deselection for transfer and how it allowed the development of algorithms thanks to the analysis of data generated during the past decade. A description of the current state of TLT is provided and the question whether to share TLT data with patients is tackled. Besides, the paper presents the non-clinical/biological interests and benefits of having TLT in IVF laboratory, which emcompass training/teaching, quality control and the management of staff time and work-flow. Overall these recommendations are mostly based on clinical and technical expertise. The paper provides technical advice, but leaves any decision on whether or not to use TLT to the individual centres.

SELECTED ORAL COMMUNICATIONS

SESSION 17: CELLULAR CHARACTERISTICS OF EMBRYO DEVELOPMENT

06 July 2020 Parallel 1 15:15 - 16:30

O-063 Prolactin receptor expression and its role in cytoskeletal reorganisation in focal adhesion in human blastocysts

K. Ezoe[1], T. Miki[1], A. Yabuuchi[1], T. Kobayashi[2], K. Kato[2]

[1]Kato Ladies Clinic, R&D Division, Tokyo, Japan ; [2]Kato Ladies Clinic, Gynecology, Tokyo, Japan

Study question: What is the gene expression pattern of prolactin receptor (PRLR) in human pre-implantation embryos and what are its functions during embryonic development and adhesion process?

Summary answer: Human embryos express PRLR at the morula and blastocyst stages and PRLR signalling stimulates blastocyst adhesion by promoting integrin-based focal adhesions.

What is known already: Predecidualisation is one of the initial processes in endometrial stromal cell differentiation that occurs during embryo implantation and placentation. During this process, the decidual cells express prolactin (PRL), a decidual marker gene, prior to implantation. PRL is thus thought to influence preimplantation development and the subsequent blastocyst implantation in the uterus. It has been reported that PRL and PRLR are not expressed in human embryos at the eight-cell stage. However, their expression patterns at stages further along the first cell lineage and implantation are poorly understood.

Study design, size, duration: A total of 318 discarded human vitrified four-cell stage embryos donated for research by consenting couples were used in this study. The study was approved by the Institutional Review Board. The embryos were randomly allocated into two groups to be cultured in medium with (n = 125) or without PRL (n = 193). The rates of blastocyst development and adhesion, outgrowth area, cytoskeletal reorganisation, and nascent adhesion formation were compared between the groups.

Participants/materials, setting, methods: Human vitrified-warmed embryos were cultured to the blastocyst stage in SAGE 1-Step medium with or without 100 mg/ml of PRL. The PRL and PRLR expressions in embryos at the four-cell, eight-cell, morula, and blastocyst stages were assessed by quantitative RT-PCR and immunofluorescence staining. The blastocysts were plated on fibronectin-coated dishes and cultured for 96 h to evaluate outgrowth competence. The expression of epithelial-to-mesenchymal transition (EMT)- and focal adhesion-related genes in blastocysts were analysed.

Main results and the role of chance: PRLR mRNA expression increased significantly after embryo compaction and blastulation. The increased expression levels of the PRLR protein at the morula and blastocyst stages were also demonstrated by immunofluorescence staining. The supplementation of the embryo culture medium with PRL did not improve the rate of embryonic development to the blastocyst stage and their morphological grade. On the contrary, blastocyst outgrowth was significantly increased in embryos cultured with PRL. The phosphorylation of JAK2, downstream of the prolactin receptor family, was markedly higher in the PRL-treated embryos than in those cultured without PRL. Furthermore, the mRNA expression of ezrin-radixin-moesin proteins, which organise the cortical cytoskeleton by linking filamentous actin to the apical membrane of cells during EMT, was stimulated by the activation of PRL-JAK2 signalling. The expression of EMT-related genes, such as transforming growth factor β1, snail1, and twist1, was also promoted by PRL treatment. PRL-treated embryos exhibited higher mRNA expression of ITGA3, ITGB1, ITGAV, and ITGB3 than non-treated embryos did. Focal adhesion kinase and paxillin are recruited by integrins and are involved in cytoskeletal and adhesion assembly and organisation. There were more nascent adherent cells expressing focal adhesion kinase and paxillin in PRL-treated embryos than in non-treated embryos.

Limitations, reasons for caution: The results may vary between *in vivo* and *in vitro* conditions. Further clinical studies are thus required to explore the clinical efficacy of PRL supplementation in the culture. This can be done by assessing the pregnancy outcomes after a single blastocyst transfer.

Wider implications of the findings: To our knowledge, this is the first report demonstrating PRLR expression in human embryos after compaction and showed that culture medium supplementation with PRL improves blastocyst adhesion by promoting EMT and integrin-based focal adhesions. Therefore, PRL treatment during embryo culture would be advantageous for improving pregnancy outcomes following blastocyst transfer.

Trial registration number: not applicable

O-064 Histone variant H3.3 chaperone complex, Hira, is essential for male pronucleus formation in mouse and human

R. Smith[1], A. Saunderson[2], S. Pickering[3], J. Tait[4], J. Thong[3], R. Anderson[4], C.J. Lin[4]

[1]University of Edinburgh, MRC Centre for Reproduction Health, Edinburgh, United Kingdom ;
[2]Royal Infirmary of Edinburgh, Simpson Centre for Reproductive Health, Edinburgh, United Kingdom ;
[3]Royal Infirmary of Edinburgh, Edinburgh Fertility and Reproductive Endocrine Centre, Edinburgh, United Kingdom ;
[4]University of Edinburgh, MRC Centre for Reproductive Health, Edinburgh, United Kingdom

Study question: Is loss-of-function of H3.3 chaperone Hira complex accountable for the abnormal single pronucleus (1PN) phenotype?

Summary answer: H3.3 Chaperone Hira complex is essential for male pronucleus formation in both mice and humans.

What is known already: The Hira complex comprises Hira, Cabin1, and Ubn1. In mouse, it is responsible for H3.3 incorporation into the male genome, chromatin reconstruction, and male pronucleus formation after fertilisation. Hira-mutant zygotes show a 1PN phenotype due to failure of formation of the male pronucleus. An abnormal 1PN phenotype is commonly found following assisted reproductive technologies (ART) in IVF clinics: however, the etiology is unknown and no investigation of whether the other subunits in the complex are required for zygote formation in mouse or human has been carried out.

Study design, size, duration: Mouse model: We have generated new loss-of-function mouse models of Hira, Cabin1, and Ubn1 respectively.

Human samples: An HFEA Research Licence allowed us to collect abnormal one-pronucleus zygotes from the Edinburgh Fertility and Reproductive Endocrine Centre, during 2018-2019. We obtained consent from 144 couples and collected 21 1PN zygotes from 16 patients.

Participants/materials, setting, methods: We used mouse models to conditionally knockout Hira and Cabin1 in oocytes using Zp3-Cre, and morpholino microinjection to study the knockdown of Ubn1. We studied the consequences of the loss-of-function on the formation of the 1PN phenotype. Embryos were generated using a combination of ART (microinjection, IVM/IVF/IVC). Histology (H&E, IHC), cytological (confocal imaging) and molecular analyses (qRT-PCR, IF, Proximity Ligation Assay) were then applied.

Human zygotes had IF and confocal imaging applied to them.

Main results and the role of chance: Results of mouse studies: We demonstrated that all the subunits of the Hira complex are maternal factors present in oocytes, and they are incorporated into male chromatin after fertilisation and interact with each other. It is important to note that loss-of-function Hira complex oocytes formed abnormal 1PN after fertilisation.

Results of Human studies: In the human abnormal 1PN zygotes we collected, we observed that Hira chaperone molecules failed to incorporate into male

chromatin. This finding is based on the detection of IF signal in male chromatin [100% of Hira (n=4); 100% of Ubn1 (n=4), and 50% of Cabin1 (n=4)]. Since the human data agreed with the mouse data, we proposed that the mechanism of action is likely conserved.

Limitations, reasons for caution: We obtained consent from only 144 couples, and collected 21 1PN zygotes. Ethical and HFEA regulations prevented us from collecting normal 2PN human zygotes. However, we were able to collect morphological abnormal but cytological normal samples as controls to demonstrate that Hira incorporates into male chromatin in the human zygote.

Wider implications of the findings: The Hira complex is critical during fertilisation in both mice and humans. The feasibility of rescuing 1PN zygotes using overexpression or nuclear transfer approaches as an alternative to the 3 parent-IVF strategy could potentially be developed into a novel personalised medicine for patients who have previously produced1PN zygotes after ART.

Trial registration number: Not applicable. HFEA Research Licence Reference R0204

O-065 The telomere link between extended fertility and longevity

J. Michaeli[1], K. Rotshenker Olshinka[1], N. Srebnik[1], R. Smoom[2], N. Serruya[2], S. Yanai[2], O. Michaeli[2], T. Eldar-Geva[1], Y. Tzfati[2]

[1]Shaare Zedek Medical Center, IVF Unit- Obstetrics and Gynecology, Jerusalem, Israel ;
[2]The Hebrew University of Jerusalem, Department of Genetics- The Silberman Institute of Life Sciences, Jerusalem, Israel

Study question: Is extended fertility associated with longer telomeres?

Summary answer: Extended fertility is associated with longer leukocyte telomere length suggesting a novel biomarker of oocyte quality.

What is known already: Telomeres are nucleoprotein complexes that protect the ends of eukaryotic chromosomes. Telomeres gradually shorten with age and eventually cause aging-related pathologies. Longer telomeres are associated with longevity. Telomeres have a cardinal role in meiosis and the development of germ cells. Many demographic studies correlated late reproduction with general health and longevity. We hypothesized that telomere length provides a link between extended female fertility and longevity. Furthermore, while anti-mullerian hormone (AMH) levels indicate the number of remaining follicles, we hypothesized that telomere length might indicate the quality of the oocytes and the ability to conceive and deliver a genetically-healthy offspring.

Study design, size, duration: A prospective experimental study evaluating: (1) leukocyte mean telomere length and AMH levels in 30 women age 43-48y who naturally conceived and delivered healthy babies, and 30 age-matched controls, previously fertile, who do not use contraceptives but failed to conceive after the age of 41y; (2) leukocyte mean telomere length in 20 women age 30-35y who delivered their first child compared to 20 age-matched women who delivered their 6^{th} to 10^{th} child. Study conducted 2018-2019.

Participants/materials, setting, methods: Blood samples obtained (1) study group: at delivery and 5-6 months later, and from the control group, and (2) at delivery of the first or $\geq 6^{th}$ child. Erythrocytes were lysed and high molecular weight genomic DNA was extracted from leukocyte by a standard proteinase K/phenol method. Average telomere length measured by Southern blot, analyzed by *TeloTool* software. AMH levels were measured (in the study group, 5-6 months postpartum) by an automated assay (Elecsys Cobas, Roche).

Main results and the role of chance: In study 1, mean age, 45y, and demographic characteristics were similar between the study and control groups. Average telomere length in the study group was 9700 bp, significantly longer than that of the control group, 9270 bp ($p<0.05$). When plotting telomere length according to parity, in women with less than 9 children, the difference in telomere length between cases and controls increased to over 1000 bp ($p=0.009$). While in the subpopulation of women with ≥9 children, the difference was insignificant. This led us to examine the immediate effects of pregnancy and delivery, and the long-term effect of parity on telomere length (study 2). Both short and long term effects were found insignificant. AMH levels were similar in the study and control groups (averaged 0.80 and 0.82 ng/ml, respectively). Our results indicate clear correlation between extended fertility and longer telomeres, particularly in the subpopulation of women with up to 8 children, which is unlikely to be found by chance ($p=0.009$). We propose that in this population of women, the quality of the oocytes, reflected by longer telomeres, is a major factor enabling successful conception. In women with more children, other factors compensate for the declined oocyte quality typical to their age.

Limitations, reasons for caution: We have measured telomere length in leukocytes, and it remains to be shown whether it reflects oocyte telomere length. In addition, the association of telomere length with oocyte quality and infertility should be studied more directly in a subfertile population of women.

Wider implications of the findings: Our findings biologically support previous demographic reports and explain the known link between extended fertility and longevity. We suggest that longer telomeres, associated with extended fertility, reflect improved general health and reproductive fitness. We propose that telomere length should be further explored as a possible novel biomarker of oocyte quality.

Trial registration number: Shaare Zedek Medical Center ethical committee approval number: 0145-16-SZMC

O-066 Cytoskeleton, ultrastructure and viability of human biopsied embryos on day 3 vs day 5 following vitrification

A. Chatzimeletiou[1], N. Petrogiannis[2], A. Sioga[3], A. Makedos[1], M. Filippa[2], B. Tarlatzis[1], E. Kolibianakis[1], A. Handyside[4], G. Grimbizis[1]

[1]Aristotle University Papageorgiou Hospital, IVF Unit, Thessaloniki, Greece ;
[2]Naval Hospital of Athens, IVF Unit, Athens, Greece ;
[3]Aristotle University, Embryology Laboratory, Thessaloniki, Greece ;
[4]Illumina, Illumina, Cambridge, United Kingdom

Study question: Are there any differences in viability, cytoskeletal abnormalities and mitochondrial and other organelles' structure/ number/ function amongst embryos biopsied on day3 vs day5 following vitrification?

Summary answer: No statistically significant differences are observed in viability, spindle/chromosome configurations and the ultrastructure of human embryos biopsied on day 3 vs day 5 following vitrification.

What is known already: Most studies in human biopsied vitrified embryos concentrate on assessing the success of the procedure on survival rates and clinical outcomes following transfer. Indeed several clinical trials have confirmed promising results. Limited studies have however examined the effects of biopsy and vitrification on spindle structure/chromosome alignment in human embryos, and only in animal models cell viability using fluorescent markers and the ultrastructure of the cellular organelles in vitrified biopsied embryos have been investigated in detail. This is the first study to examine in human embryos biopsied at 2 different stages cell viability, cytoskeleton and ultrastructure before and after vitrification.

Study design, size, duration: 240 Day3 biopsied embryos that developed to blastocysts but were rejected for transfer following PGT-M or PGT-A were divided into 2 groups: A) 120 blastocysts treated for Viability, Cytoskeletal and TEM analysis(fresh n=20,n=20,n=20 and following vitrification/warming n=20,n=20,n=20), B) 120 embryos were rebiopsied at the blastocyst stage and treated for Viability, Cytoskeletal and TEM analysis(fresh n=20,n=20,n=20 and following vitrification/warming n=20,n=20,n=20). Also, 60 vitrified blastocysts biopsied only on Day5 were treated for Viability, Cytoskeletal and TEM analysis.

Participants/materials, setting, methods: Viability was assessed by CFSE/PI staining. Cytoskeletal analysis by confocal laser scanning microscopy was performed following immunostaining with α-tubulin, γ-tubulin and acetylated-tubulin antibodies in combination with DAPI or /PI. For ultrastructure assessment by transmission electron microscopy(TEM), blastocysts were fixed in gluteraldehyde, incubated in osmium, aqueous uranyl acetate, dehydrated through ethanol series and immersed in Epon. The Ultrathin lead citrate stained sections were examined in a JEOL-TEM-2000-FXII microscope. The study was conducted in an academic hospital.

Main results and the role of chance: Viability staining with CFSE/PI before vitrification revealed that all blastocysts biopsied on day5 had damaged cells at the position of cutting (range 4-10cells), while 95% of the day3 biopsied embryos that developed to the blastocyst stage had 0 cells damaged at the herniating site. Following vitrification and subsequent warming, additional PI stained cells (range 5-20) were evident in other parts of the day5 biopsied blastocysts and in similar incidence to vitrified blastocysts that were biopsied on day3. Cytoskeletal analysis by confocal laser scanning microscopy revealed that the majority of spindles examined in both fresh and vitrified biopsied embryos were normal. However in the vitrified groups a higher incidence of spindle abnormalities was observed which

was not significantly different between Day3 biopsied embryos (33/98 (33.7%) abnormally shaped spindles and 5/98 (5.1%) multipolar and monopolar spindles and day5 biopsied embryos (42/128 (32.8%) abnormally shaped spindles and 7/128 (5.5%) multipolar and monopolar spindles (p>0.05). Transmission electron microscopy (TEM) analysis revealed similar ultrastracture between the day 3 and day 5 biopsied embryos before vitrification. However, compared to fresh blastocysts vitrified blastocysts had more characteristic lipofuscin droplets (representative of apoptosis) and a higher number of vacuoles and distension of mitochondria.

Limitations, reasons for caution: The blastocysts used in this study were all diagnosed with either chromosomal abnormalities or single gene defects following PGT-A or PGT-M.

Wider implications of the findings: This is the first study to compare viability, ultrastructure and spindle/chromosome configurations in day3 and day5 biopsied embryos following vitrification. The similarities observed in ultrastucture reflect similar patterns of metabolism, while lipofuscins indicate 'rescue' processes for the embryos, which in their attempt to fully recover following vitrification, eliminate damaged/abnormal cells.

Trial registration number: not applicable

O-067 Smooth endoplasmic reticulum clusters in oocytes influence pronuclear behaviour and morphokinetics at early-cleavage stage but have no negative impact on embryonic and pregnancy outcomes

S. Ueno[1], T. Miki[2], U. Kazuo[1], T. Okimura[1], A. Yabuuchi[2], T. Kaobayashi[3], K. Kato[3]

[1]Kato Ladies Clinic, IVF Laboratrory, Tokyo, Japan ;
[2]Kato Ladies Clinic, R&D division, Tokyo, Japan ;
[3]Kato Ladies Clinic, Gynaecology, Tokyo, Japan

Study question: Do smooth endoplasmic reticulum clusters (sERCs) influence pronuclear behaviour at fertilisation, morphokinetics, blastocyst development, and live birth following single embryo transfer (SET)?

Summary answer: Smooth ERCs influence pronuclear behaviour at fertilisation and morphokinetics at early-cleavage stage, but have no adverse impact on embryonic and pregnancy outcomes.

What is known already: sERC-positive (sERC+) oocytes and/or cycles in which sERC+ oocytes were retrieved are reported to be associated with poor clinical outcomes following IVF-ET. However, the effects of sERCs on embryonic and pregnancy outcomes remain unclear. The endoplasmic reticulum (ER) is a major internal storage site of calcium ions, and the ions stored in the ER should be mobilized properly during fertilisation for further embryonic development. Therefore, it is hypothesized that sERCs may be associated with aberrant pronuclear (PN) behaviour at fertilisation and morphokinetics during embryo development; however, the number of studies supporting this hypothesis is limited.

Study design, size, duration: A retrospective study of 6,010 cycles (mean age: 38.3±3.5 years old), including 294 cycles with sERC+ cycles was conducted between 2014 and 2018. Study 1: PN size and behaviour at fertilisation, direct cleavage rate in 1st cytokinesis (DC-1), morphokinetics during embryo development, and blastocyst utilisation rates were compared between sERC+ and, sERC- oocytes obtained from sERC- cycle. Study 2: Live birth rates (LBRs) following SET were compared between sERC+ and, sERC- oocytes from sERC- cycle.

Participants/materials, setting, methods: Oocytes were inseminated by ICSI and were cultured in either a dry incubator (ASTEC, Japan) or a time-lapse incubator (Vitrolife, Sweden). Morhpokinetics were analysed using EmbryoViwer (Vitrolife). Wilcoxon rank sum test and chi-square test were used to compare the PN size, DC-1 rate, and morphokinetics between sERC+ and sERC- oocytes. Chi-square test and multivariate logistic regression analysis were used to compare blastocyst utilisation and LBRs at >22 weeks of pregnancy between sERC+ and sERC- oocytes.

Main results and the role of chance: Study 1: The time (hours) required from insemination to PN appearance (male: 4.27±1.28 vs. 4.49±1.47, female: 5.10±1.43 vs. 5.30±1.55), PN alignment (7.90±2.51 vs. 8.65±2.95), PN fading (22.3±3.7 vs. 23.4±4.2), 2-cell (25.1±4.2 vs. 26.1±4.3), 3-cell (35.1±5.4 vs. 35.6±6.2), and 4-cell (37.4±6.9 vs. 38.0±6.5) stages was significantly faster in the sERC+ oocytes than in the sERC- oocytes ($P < 0.05$). The difference in PN size (between male and female) immediately before PN fading was significantly larger in the sERC+ oocytes than in the sERC- oocytes (105.5 ± 106.1μm^2 vs, 72.8 ± 67.2μm^2, $P < 0.05$). However, there were no significant differences in DC-1 rates and the time from insemination to reaching the 5-cell, 8-cell, blastulation, and expanding blastocyst stages between sERC+ and sERC- oocytes. No correlation was observed between sERC+ status and blastocyst utilisation rate [sERC+ oocyes: 46.2%, 96/209, sERC- oocytes: 48.7%, 2,111/4,331, adjusted odds ratio: 1.01, 95% confidence interval (CI): 0.75-1.35]. Study 2: The LBRs following SET in sERC+ and sERC- oocytes were 21.7% (10/46) and 18.6% (546/2942), respectively. There was no significant correlation between the sERC+ status and LBR (adjusted odds ratio: 1.35, 95%CI: 0.79-2.42). Furthermore, all of the babies derived from sERC+ oocytes were healthy.

Limitations, reasons for caution: The retrospective design and small sample size are the major limitations. The rate of sERC+ oocytes was very low during the study period. Therefore, these were the only cases we could identify. Further studies are needed to explore the mechanism by which sERCs influence PN behaviour and morphokinetics.

Wider implications of the findings: Our results demonstrated that sERC did not adversely affect embryonic and pregnancy outcomes. Therefore, it was suggested that sERC+ oocytes could be used for IVF treatment. We also found that sERCs correlated with PN behaviour and morphokinetics in early-cleavage stage. Further detailed investigations are required to confirm these findings.

Trial registration number: not applocable

SELECTED ORAL COMMUNICATIONS

SESSION 18: CELLULAR AND MOLECULAR MARKERS OF OVARIAN AGEING

06 July 2020 Parallel 2 15:15 - 16:35

O-068 Which accumulation is better in poor ovarian responders stratified by POSEIDON, egg or blastocyst?

T.H. Chuang[1], J.Y. Hsieh[1], C.T. Kuo[1], M.J. Lee[1], H.L. Wang[1], H.H. Lai[1]

[1]Stork Ladies Clinic, Stork Fertility Center, Hsinchu, Taiwan R.O.C.

Study question: Which accumulation is better in poor ovarian responders stratified by POSEIDON, egg or blastocyst?

Summary answer: Both egg accumulation or blastocyst accumulation achieve comparable clinical outcomes in poor ovarian responders.

What is known already: The patients have poor responses to ovarian stimulation (POR) generally resulted in low quantity of retrieved oocytes, fewer available embryos, and thus reduced pregnancy rate. Attributing to the improvement of the vitrification technique in human eggs and embryos, accumulation of eggs/embryos becomes an effective and safe way to increase the number of available embryos and the opportunity of a successful pregnancy.

Study design, size, duration: In this retrospective study, 955 oocyte pick-up (OPU) cycles were involved from January 2017 to October 2019, consisting of 409 cycles from 110 patients with egg accumulation (EAc) and 546 cycles from 172 patients with blastocyst accumulation (BAc). The definition of POR was based on the POSEIDON criteria (Humaidan et al., 2016). These patients decided to undergo one of the accumulative strategies by themselves after consulting their doctors about the pros and cons.

Participants/materials, setting, methods: The inclusion criteria of patients were age ≥35 years, AMH <1.2 ng/mL, and retrieving more than one oocyte in each OPU cycle. The metaphase II (MII) oocytes were cryopreserved in the EAc group, and the blastocysts grading above BC according to Gardner system were cryopreserved in the BAc group via vitrification (Cryotech®, Japan). The clinical outcomes were calculated in the patients underwent frozen-thawed blastocyst transfer.

Main results and the role of chance: The mean retrieved MII oocytes per case (EAc: 8.2±4.2, BAc: 7.4±6.2, $p<0.05$) and the mean obtained blastocyst per case (EAc: 3.3±2.6, BAc: 2.9±2.6, $p<0.05$) were significantly higher in the EAc group. In contrast, significantly higher fertilization rate (EAc 71.7%, BAc: 76.0%, $p=0.03$) was observed in the BAc group. No difference was found in good blastocyst formation rate (EAc: 50.3%, BAc: 51.2%, $p=0.73$) between the two groups. Of reproductive outcomes, the biochemical pregnancy rate (BPR), clinical

pregnancy rate (CPR), and implantation rate (IR) between the EAc and the BAc groups were comparable: [BPR] 52.9% vs. 58.1% (p=0.60); [CPR] 37.1% vs. 27.9% (p=0.29); [IR] 22.0% vs. 26.0% (p=0.53). Of PGT-A transfer cycles, there was no significant difference between the two groups. ([IR] 40.0% vs. 37.7%, p=1.0)
Limitations, reasons for caution: The retrospective nature is the main limitation of this study.
Wider implications of the findings: Though the laboratory outcomes showed differences between the patients with embryo and egg accumulation, the clinical outcomes of cryotransfer displayed comparable in the two populations.
Trial registration number: not applicable

O-069 Human ovarian ageing can be caused by Reactive Oxygen Species (ROS)-induced damage in primordial follicles

M. Smits[1], R. Wüst[2], F. Dijk[3], S. Dorrestein[1], M. Kleijn[1], M. Goddijn[1], R. Houtkooper[4], S. Mastenbroek[1], G. Hamer[1]

[1]Amsterdam UMC- University of Amsterdam, Center for Reproductive Medicine, Amsterdam, The Netherlands ;
[2]VU University, Department of Human Movement Sciences, Amsterdam, The Netherlands ;
[3]Amsterdam UMC- University of Amsterdam, Pathology, Amsterdam, The Netherlands ;
[4]Amsterdam UMC- University of Amsterdam, Laboratory Genetic Metabolic Diseases, Amsterdam, The Netherlands

Study question: Can ovarian ageing be caused by life-long oxidative phosphorylation, and therefore reactive oxygen species exposure, in oocytes and granulosa cells?
Summary answer: Oxidative phosphorylation, and reactive oxygen species (ROS)-induced damage, are visible during all stages of folliculogenesis, suggesting life-long ROS-exposure could indeed contribute to ovarian ageing.
What is known already: Oocyte quality declines with increasing female age. The reason for this is unknown. Oogenesis mainly occurs before birth after which oocytes have to remain dormant in primordial follicles until they are ovulated, a process that may take decades. Damage caused by ROS released from mitochondria during oxidative phosphorylation might be involved in the decline in oocyte quality. Although based on little experimental evidence, the current thinking is that, to prevent generation of ROS, primordial follicles only use glycolysis for energy supply. We have now assessed mitochondrial activity and ROS-induced damage in oocytes and granulosa cells throughout all stages of folliculogenesis.
Study design, size, duration: Using the Dutch Pathology Registry (PALGA), we collected ovarian biopsies stored in the Netherlands between 1991 and 2018. Biopsies were excluded in case of ovarian malignancies, polycystic ovarian syndrome or preventive resection due to *BRCA* mutation carrier. In total, 39 ovarian biopsies of individual patients, ranging in age from 18 to 45 years were received, of which 34 contained at least one follicle.
Participants/materials, setting, methods: Ovarian biopsies were used for high resolution microscopic analysis of mitochondrial structure, activity and ROS-induced damage to proteins, lipids and DNA. Antibodies used were: TOMM20, anti-phosphorylated pyruvate dehydrogenase (PDHA), anti-3-Nitrotyrosin, anti-4-Hydroxynonenal and anti-8-Hydroxy-2'-deoxyguanosine. Deconvoluted Z-stack images of follicles at different developmental stages were taken on a Leica SP8 fluorescence microscope, and analyzed by Leica Application Suite X. IBM SPSS Statistics 25.0 was used for statistical analysis.
Main results and the role of chance: For the first time, we showed phosphorylated pyruvate dehydrogenase activity in oocytes and granulosa cells in all stages of follicular development: from primordial follicles to tertiary follicles. This indicates that pyruvate is actively converted into acetyl-CoA, and transported into the citric acid cycle, rather than used for glycolysis. We observed ROS-induced cellular damage in oocytes of primordial follicles, in women of all reproductive ages. No age-related differences in immunofluorescence intensity ratios between mitochondria (TOMM20 antibody intensity) and mitochondrial activity (phosphorylated PDHA antibody intensity) were observed. But ROS-induced damage in lipids and proteins seemed to show an increasing trend with age, although not statistically significant. The accumulation of ROS damage could impair oocyte quality, leading to decreased chances of becoming pregnant, and increased chances of pregnancy loss as seen in women of advanced maternal age.
Limitations, reasons for caution: Oxidative phosphorylation and ROS damage were demonstrated in all stages of follicle development, but sample size, in combination with the microscopy techniques used, limited our statistical analyses based on maternal age groups.
Wider implications of the findings: We demonstrated that oocytes and granulosa cells at all stages of folliculogenesis have active mitochondria and generate ROS, contributing to protein and lipid damage, which can accumulate during ageing. Future studies on the contribution of oxidative phosphorylation to the decline in fertility of women of advanced maternal age seem warranted.
Trial registration number: not applicable

O-070 Serum dehydroepiandrosterone sulphate (DHEA-S) concentration is a predictive factor for clinical pregnancy in women with poor ovarian response undergoing in vitro fertilization (IVF)

A. Fuentes[1], K. Sequeira[1], A. Muñoz[1], A. Tapia-Pizarro[1]

[1]IDIMI - Hospital San Borja Facultad de Medicina- Universidad de Chile, Reproduccion Humana, Santiago- RM, Chile

Study question: Is it possible to predict clinical pregnancy rate (CPR) in women with poor ovarian response (POR) undergoing IVF?
Summary answer: Serum Dehydroepiandosterone sulfate (DHEA-S) concentration showed to predict clinical pregnancy in women with POR undergoing IVF
What is known already: Serum DHEA-S levels have been shown to decrease with age. In parallel, the probability of pregnancy also decreases. The purpose of this study was to identify a minimum serum DHEA-S level that correlates with acceptable CPR in women with POR undergoing IVF
Study design, size, duration: Prospective cohort study involving 70 women followed at the tertiary referral center, IDIMI, Universidad de Chile, from 2017-2018
Participants/materials, setting, methods: A cohort of women with POR (n = 50) and a control group (n = 20) undergoing IVF were studied. Serum levels of DHEA-S, AMH, FSH, estradiol as well as Antral follicle count (AFC) on day 3 of ovarian stimulation were assessed. DHEA-S concentrations in follicular fluid at the time of oocyte pick-up were also measured. Clinical pregnancy outcomes were correlated with the assesed parameters. Correlations and ROC analyses were perfomed to predict clinical pregnancy
Main results and the role of chance: There was no difference between the two groups in day 3 FSH and estradiol. Mean age and duration of ovarian stimulation were significantly higher in women with POR than controls (35.7±0.55 *vs.* 32.84±1,19 and 11.28±0.27 *vs.* 10.31±0.36 respectively) while day 3 AFC, Serum DHEA-S, Follicle-to-Oocyte Index (FOI) and serum AMH concentrations were significantly lower in the POR group than control (6±0.36 *vs.* 12.47±1.73, 160.57±12.52 *vs.* 218. ±25.30, 0.59±0.66 *vs.* 1.42±0.22, and 0.76±0.14 *vs.* 2.50±0.72; respectively). Serum DHEA-S concentration strongly correlated with those in follicular fluid (Pearson index = 87.5%). The ROC analysis for DHEA-S *vs.* pregnancy showed an area under the curve of 81.1% with a sensitivity of 87.5% and a specificity of 80.3 % with a cutoff value of 221.4 µg/dL
Limitations, reasons for caution: This prospective pilot study significantly implicates endogenously circulating levels of DHEA-S in the clinical pregnancy rates. Although our study has a power of 81%, more systematic studies/RCTs with larger sample size are required to confirm circulating DHEA-S levels as an efficient and robust predictor of clinical pregnancy rates
Wider implications of the findings: This study underlines the significance of optimum levels of endogenous DHEA-S for successful implantation. Our results suggest that the so called adrenopause of any origin seems to be associated with poor results in IVF. This information can be crucial during the pre-IVF counseling of these women
Trial registration number: not applicable

O-071 Longevity pathways are involved in human ovarian ageing

G. Janssens[1], M. Smits[2], M. Goddijn[2], G. Hamer[2], R. Houtkooper[1], S. Mastenbroek[2]

[1]Amsterdam UMC- University of Amsterdam, Laboratory Genetic Metabolic Diseases, Amsterdam, The Netherlands ;
[2]Amsterdam UMC- University of Amsterdam, Center for Reproductive Medicine, Amsterdam, The Netherlands

Study question: Are gene expression pathways known to be involved in general aging, particularly longevity pathways, also involved in human ovarian ageing?
Summary answer: Growth, metabolism, and cell cycle progression related pathways that are known to be involved in general ageing appear to play a role in ovarian ageing.
What is known already: Oocyte quality declines with advancing maternal age, a process generally referred to as ovarian ageing. Genetic pathways that modulate the rate of general, somatic cell, ageing have been researched intensively. Pro-longevity genes slow down the process of ageing and increase life span, while anti-longevity genes accelerate ageing and therefore decrease life span. Ovarian ageing does not follow the same time line as general ageing. It is not known whether generally recognized longevity genes also play a role during ovarian ageing. Identification of ovarian ageing pathways can lead to new hypotheses and possible treatment options for subfertility caused by ovarian ageing.
Study design, size, duration: We analyzed a dataset of individual gene expression profiles of 38 Germinal Vesicle (GV) oocytes of 38 women aged between 25 and 43 years old. Correlations between female age (calendar age and biological age, i.e. dosage of follicle stimulating hormones, antral follicle count (AFC)) and longevity pathways were investigated using a database of known longevity pathways.
Participants/materials, setting, methods: Transcripts of 38 GV oocytes were used for individual gene expression analysis. R version 3.5.1 was used to process and analyze data. GeneAge database build 19 was used to obtain mouse ageing related genes. Human to mouse orthologs were obtained using the R package biomaRt. Correlations and significance between gene expression data and ages was tested for using Pearson's product moment correlation coefficient using ranked expression data. Distributions were compared using a two-sample Student's t-tests.
Main results and the role of chance: 46 anti-longevity genes showed a positive correlation with female calendar age and FSH dosage administered during ICSI treatment and a negative correlation with AFC. 84 pro-longevity genes were negatively correlated with calendar age and FSH dosage, while positively correlating with AFC.

In general, pro- and anti-longevity genes changed in opposing directions with advancing maternal age in oocytes in a manner that, in somatic cells, represents ageing and a limited life span, and in oocytes could reflect the potential or the quality of the oocyte.

Notably, the anti-longevity genes include many 'growth' related genes involved in the MTOR pathway, such as *EIF5A2*, *EIF3H*, *EIF4E*, and *MTOR*. The pro-longevity genes include many cell cycle progression related genes involved in DNA damage repair (e.g. *XRCC6, ERCC2,* and *MSH2*) or cell cycle checkpoint regulation (e.g. *ATM*, *BRCA1*, *TP53*, *TP63, TP73* and *BUB1B*).
Limitations, reasons for caution: Using mature oocytes instead of GV oocytes may potentially provide different results. No correction for multiple testing was carried out because a small set of longevity related genes were selected a priori for the analysis.
Wider implications of the findings: Growth, metabolism, and cell cycle progression related pathways that are known to be involved in general ageing appear to play a role in ovarian ageing. We suggest that interventions known to modulate these processes could benefit women suffering from ovarian ageing.
Trial registration number: not applicable

O-072 Investigating apoptotic, inflammatory and growth markers in the follicular fluid of poor responders undergoing natural cycle IVF treatment

N. Nitsos[1], K. Sfakianoudis[2], P. Tsioulou[3], A. Glava[3], E. Maziotis[3], P. Giannelou[3], S. Grigoriadis[3], A. Rapani[3], A. Nezos[3], K. Pantos[2], M. Koutsilieris[3], N. Vlahos[4], G. Mastorakos[5], M. Simopoulou[3]

[1]Genesis Athens Clinic, Microbiology Laboratory, Athens, Greece ; [2]Genesis Athens Clinic, Assisted Conception Unit, Athens, Greece ; [3]National and Kapodistrian University of Athens, Physiology, Athens, Greece ; [4]Aretaieion Hospital- Medical School- National and Kapodistrian University of Athens, Second Department of Obstetrics and Gynecology, Athens, Greece ; [5]Aretaieion Hospital- Medical School- National and Kapodistrian University of Athens, Unit of Endocrinology- Diabetes Melitus and Metabolism- 2nd Department of Obstetrics and Gynecology- Aretaieion Hospital- Medical School- National and Kapodistrian Uni,

Study question: Do poor responders present with different levels of cfDNA concentration and integrity, as well different levels of inflammatory and growth markers in follicular fluid?
Summary answer: Poor responders present with similar cfDNA levels and integrity, compared to normal responders in natural cycles, albeit with lower follicular fluid levels of G-CSF.
What is known already: Embryo developmental potential is associated with oocyte quality and maturation status. Follicular fluid includes a wide pallet of hormones, growth factors and members of the Transforming Growth Factor-beta superfamily, interleukins, cytokines, reactive oxygen species, anti-apoptotic factors, peptides, amino-acids, sugars, and prostanoids, in conjunction with circulating cell-free DNA. These markers observed in follicular fluid affect the ovarian folliculogenesis process, and subsequent embryo development and pregnancy outcome. A better understanding of the pathophysiological processes involved in poor ovarian response (POR) is of added value.
Study design, size, duration: A total of 88 patients undergoing IVF, were enrolled in this prospective single-center observational study, from September 2018 to July 2019. Forty-four of these patients were identified as poor responders according to Bologna Criteria, whereas the remaining 44 were classified as normal responders. All patients underwent natural cycles with fresh embryo transfers. Clinical pregnancy was similarly recorded.
Participants/materials, setting, methods: Follicular fluid was collected as part of the oocyte retrieval process. Levels of cfDNA were quantified via Q-PCR employing the ALU115 and ALU247 primers, associated with necrotic and apoptotic and necrotic events respectively. The Q247/Q115 ratio represents cfDNA integrity. Follicular fluid levels of inflammatory markers-Interleukin-15 (IL-15) and Corticotropin-Releasing Hormone (CRH)- and of growth factors – Insulin-like Growth Factor 1 (IGF-1), Vascular Endothelial Growth Factor (VEGF) and Granulocyte Colony-Stimulating Factor (G-CSF) were evaluated via ELISA.
Main results and the role of chance: In the normal responders group a positive correlation between CRH and IL-15 was established ($p<0.001$), as well as between G-CSF and progesterone ($p=0.03$). A negative correlation between E2 and cfDNA integrity was also observed ($p=0.005$). In the poor responders group a positive correlation was established between VEGF and ALU 115 ($p=0.006$) as well as ALU 247 ($p=0.01$). Negative correlation was established between VEGF and FSH ($p=0.02$). IGF-1 was negatively correlated with AMH ($p=0.04$) and CRH ($p=0.008$). A positive correlation CRH and IL-15 was also observed ($p=0.005$). Between the two groups no statistically significant difference was observed regarding the levels of IL-15, CRH, IGF-1 or VEGF. The levels of G-CSF were statistically significant higher in the normal responders' group when compared to the poor responders' group ($p=0.003$). No statistically significant difference was observed between the groups regarding cfDNA concentration or cfDNA integrity. Cycles that resulted in clinical pregnancy presented with a lower cfDNA integrity compared to the cycles not leading to clinical pregnancy both in the normal and in the poor responders' group (Normal: 0.07±0.04 vs 0.25±0.17, $p<0.001$; Poor: 0.10±0.06vs0.26±0.12 $p<0.001$ respectively). Cycles with successful blastocyst formation presented with higher cfDNA levels in the normal responders' group (0.38±0.26vs0.82±0.66 $p=0.03$).
Limitations, reasons for caution: Limitations of our study refer to the limited size of the studied population, as well as lack of data referring to the all-inclusive patients' history, namely body max index, exercise activity or smoking, which may be identified as possible sources affecting cfDNA levels.
Wider implications of the findings: Poor and normal responders present with similar levels of inflammatory, apoptotic or necrotic markers. Lower levels of G-CSF associated to poor responders indicate G-CSF's role in folliculogenesis/follicular maturation. Levels of cfDNA and integrity, correlated with clinical outcome, highlighting their role as possible biomarkers. A conclusive verdict requires larger studies.
Trial registration number: Not applicable

O-073 Transcriptome analysis of human granulosa cells after conventional controlled ovarian stimulation versus mild ovarian stimulation in poor ovarian responders

X.P. Liu[1], C.C. Zhou[1], J.J. Li[1], T.B. Wu[1], H.S. Mai[1], X.Y. Liang[1], R. Huang[1]

[1]Reproductive Medicine Center, 6th Affiliated Hospital-Sun Yat-sen University, Guangzhou, China

Study question: What is the difference on the transcriptome of human granulosa cells after mild ovarian stimulation and conventional controlled ovarian stimulation in poor responders?
Summary answer: Different stimulation protocols for poor responders induced alterations in the granulosa cells that may affect immune processes, cytokine interactions and the pathway of oocyte development.
What is known already: No consensus is available regarding the optimal decision between mild stimulation and conventional controlled ovarian stimulation (COS) protocols for poor responders. Currently, studies comparing the efficacy of COS versus mild stimulation have generally focused on clinical pregnancy outcomes. Until now, none of studies have discussed the impact of such two different stimulation regimens on the cellular physiology of follicular cells in poor responders. There is no referable evidence that based on follicular physiology to support which stimulation protocol is optimal for them.
Study design, size, duration: This is an experimental study which contained 48 poor responders according to Bologna criteria.
Participants/materials, setting, methods: Forty-eight patients who met the Bologna criteria of poor ovarian response were allocated into two groups as mild stimulation or COS. Twenty-seven patients allocated to the mild stimulation group, while 21 patients in the COS group. Granulosa cells are collected on the oocyte pick-up day, and RNA was extracted and sequenced using Illumina Hiseq technology. Some of the differently expression genes were validated by real-time quantitative PCR.
Main results and the role of chance: In summary, a total of 192 genes were up-regulated, and 233 genes were down-regulated, in the mild stimulation group compared with the conventional controlled stimulation group. Gene Ontology analysis and Kyoto Encyclopedia of Genes and Genomes analysis revealed that the differently expression genes between the two groups were mainly involved in cytokine-cytokine receptor interactions, TGF-beta signaling pathway, the biological function of regulation activity and immune response, which associated with the development of the follicle and the oocyte.
Limitations, reasons for caution: The sample size was relatively small in our study. There is a need for larger number of patients.
Wider implications of the findings: The two different ovarian stimulation protocols for poor responders cause dissimilar biological functions in granulosa cells, which have influence on the development of the oocytes and subsequent IVF outcome. Most importantly, our findings indicate that the mild ovarian stimulation may be more benefit to the competence of the oocytes.
Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 19: RIF AND ENDOMETRIAL FACTORS: DOES IT MATTER?

06 July 2020 Parallel 3 15:15 - 16:30

O-074 Efficacy of endometrial microbiome metagenomic analysis and analysis of infectious chronic endometritis on in vitro fertilization outcome in women with recurrent implantation failure

N. Iwami[1], M. Kawamata[1], N. Ozawa[1], T. Yamamoto[1], E. Watanabe[1], M. Mizuuchi[1], O. Moriwaka[1], H. Kamiya[1]

[1]Kamiya Ladies Clinic, Center of reproduction, Sapporo, Japan

Study question: Following results of endometrial microbiome metagenomic analysis (EMMA) and analysis of infectious chronic endometritis (ALICE), is there an effect post treatment for the patients with recurrent implantation failure (RIF)?
Summary answer: The clinical pregnancy rate of the patients who underwent EMMA/ALICE testing was significantly higher than that of the patients who did not undergo testing.
What is known already: Chronic endometritis (CE) is persistent endometrial inflammation mainly caused by bacterial infections. CE is found in about 30% of infertile women, and 60% of patients with RIF, so pathogenic flora identification is the first step of treatment. Using next generation sequencing (NGS) technology, EMMA/ALICE testing can determine the composition of the endometrial microbiome by analysing bacterial 16S ribosomal RNA with a focus on *Lactobacillus* population. *Lactobacillus*-dominated microbiota (LDM, defined as >90% *Lactobacillus* species) in the endometrium was reported to be associated with favorable reproductive outcome, while non-LDM (<90% *Lactobacillus* species) was found to decrease implantation, clinical pregnancy, and ongoing pregnancy.
Study design, size, duration: The prospective cohort study consisted of 134 women with RIF (defined as at least three previous failed *in vitro* fertilization (IVF)-embryo transfer (ET) attempts) from July in 2018 to October in 2019 at our infertility center. EMMA/ALICE testing were suggested to all patients who had failed ET three times or more. Ninety-eight patients underwent EMMA/ALICE (Study group) before additional transfer, 36 patients with history of RIF continued ET without both examinations (Control group).
Participants/materials, setting, methods: During the patients' luteal phase, endometrial biopsies were performed for EMMA/ALICE testing, and treatment was given based on the results. The primary outcome measure was the cumulative clinical pregnancy rate after two additional ET. Clinical pregnancy was defined by visualization of a gestational sac. Statistical analysis was performed using unpaired t-test and chi-square contingency.
Main results and the role of chance: Patients characteristics such as age, body mass index, duration of infertility, and anti-mullerian hormone (AMH) were comparable between the groups. The mean number of unsuccessful implantations were comparable between the groups (Study group: 5.33±3.03 vs. Control group: 5.22±2.60, P=0.841). The rate of the patients with the history of miscarriage was significantly higher in Study group than that of Control group (Study group: 48.0% vs. Control group: 19.4%, p<0.010). According to the results of EMMA, 44 patients (44.9%) with normal microbiota (LDM) didn't receive probiotic treatment. The other 54 patients (55.1%) received probiotic therapies. In addition, among non-LDM patients, abnormal microbiota was detected in 14 patients (14.3%) in EMMA, and ALICE detected significant amounts of pathogenic bacteria in other 6 patients. Those 20 patients had antibiotics corresponding to the detected bacteria as well as probiotic treatment. As results, the cumulative clinical pregnancy rate in Study group was significantly higher than in Control group (Study group: 54.1% vs. Control group: 27.8%, p<0.010). However, there was no significant difference in the ongoing pregnancy rate (Study group: 44.9% vs. Control group: 27.9%, p=0.073) and the miscarriage rate (Study group: 15% {8/53 cases} vs. Control group: 0% {0/10 cases}, p=0.332).
Limitations, reasons for caution: The main limitation of our study is the lack of randomization, although it is prospective. As the sample size is small, further prospective studies are needed to confirm the efficacy of EMMA/ALICE.
Wider implications of the findings: Dysbiotic microbiota could be found in patients more than half using the NGS technique. Personalized treatment recommendations based on the EMMA/ALICE results can improve IVF outcome of RIF and repeated pregnancy loss. Moreover, broad-spectrum antibiotics treatments can be avoided, reducing the physical and economic burdens on patients.
Trial registration number: Not applicable

O-075 Composition of the endometrial microbiome is associated to reproductive outcomes in IVF patients

I. Moreno[1], I. Garcia-grau[2], D. Bau[3], D. Perez-Vilarrolla[3], M. Gonzalez-Monfort[2], D. Valbuena[4], F. Vilella[5], C. Simon[6]

[1]Igenomix, Research, Boston, U.S.A. ;
[2]Igenomix, Research Department, Valencia, Spain ;
[3]Igenomix, Bioinformatics Department, Valencia, Spain ;
[4]Igenomix, Clinical Department, Valencia, Spain ;
[5]Igenomix Foundation / INCLIVA, Research Department, Valencia, Spain ;
[6]Valencia University- Harvard University, Department of Obstetrics and Gynecology, Valencia- Boston, Spain

Study question: Is there an association between the composition of the endometrial microbiota and the reproductive outcomes in infertile patients undergoing in vitro fertilization (IVF)?
Summary answer: The composition of the endometrial microbiota investigated the cycle prior to embryo transfer differs between patients with successful versus unsuccessful clinical outcome.
What is known already: The investigation of bacterial communities in the female reproductive tract using molecular methods has revealed the existence of a continuum microbiota that extends from the vagina to the upper genital

tract. Despite similarities between vaginal and uterine microbiota, the endometrial and vaginal microbiota are different in 20% of patients. The analysis of bacterial composition in endometrial microbiota during the window of implantation has shown an association of absence of pathogens and high abundance of Lactobacilli with IVF success, while the presence of pathogenic bacteria -non-Lactobacilli dominated microbiota-, is associated with poor reproductive outcomes in assisted reproductive treatments (ART).

Study design, size, duration: This is a prospective observational multicenter study including a total 452 patients. The study was performed in 13 reproductive centres located in Europe, North America, South America and Asia. The duration of the study was 30 months and the recruitment period extended for 1.5 years starting from August 4, 2017.

Participants/materials, setting, methods: Patients undergoing IVF receiving personalized embryo transfer with frozen blastocysts in a hormonal replacement therapy in whom endometrial fluid was collected at the time of the ERA test the cycle prior to embryo transfer. Taxonomic data were generated by sequencing of the 16S rRNA gene using the Ion 16S metagenomics kit (Thermofisher) and analysed using compositional data to transform scale-invariant values and avoid bias derived of technical different between batches.

Main results and the role of chance: The interim analysis in 177 patients with different clinical outcomes including no pregnancy, (n=82), ongoing pregnancy + live birth (n=69), biochemical pregnancy (n=10), or clinical miscarriage (n=16) revealed differential compositional data of the endometrial microbiota collected in the cycle prior to the embryo transfer. Based on the analysis of bacterial genera with an abundance >2-fold that the average, 29 specific taxa were identified to discriminate between the microbiota profile of women who did not become pregnant and those whose transfers resulted in live births. When the compositional data were interrogated for taxa with >2-fold abundance and filtering from those genera most differentially represented between these two categories, *Citrobacter, Haemophilus, Kocuria, Gemella, Enterococcus, Gardnerella, Atopobium* and *Aeromonas* were differentially identified discriminating between the successful or unsuccessful outcome groups. Patients that did not become pregnant presented higher abundance of *Enterococcus* and *Haemophilus*; patients with biochemical pregnancies presented increased abundance of *Enterococcus, Gardnerella, Haemophilus* and *Kocuria;* while patients suffering clinical miscarriages presented higher abundance of *Enterococcus.* The confounding factors analysed to ruled out any bias of the reported results were body mass index, age of patient, endometrial receptivity analysis (ERA) results and preimplantation genetic diagnosis of embryos.

Limitations, reasons for caution: These are interim results from 177 out of 452 patients recruited. Because the endpoint of the study was live birth, final results will only be complete when all the babies will be born.

Wider implications of the findings: The differential endometrial microbiota composition associated to different clinical outcomes in patients undergoing IVF might have profound implications for understanding the microbiology of intrauterine environment and its implication in unknown causes of infertility and implantation failure.

Trial registration number: 03330444

O-076 Twelve-month follow-up results of a randomized controlled trial studying endometrial scratching in women with one failed IVF/ICSI cycle (the SCRaTCH trial)

N. Van Hoogenhuijze[1], H.L. Torrance[1], SCRaTCH study group(2), M.J.C. Eijkemans[1], F.J.M. Broekmans[1]

[1]University Medical Center Utrecht, Gynaecology, Utrecht, The Netherlands ; [2]Radboud University Medical Centre, dr. K. Fleischer, ; Academic Medical Centre Amsterdam, dr. F. Mol, ; Isala Fertility Clinic, dr. G. Teklenburg, ; Jeroen Bosch Hospital, dr. J.P. de Bruin, ; Maastricht University Medical Centre, dr. J.E. den Hartog, ; Vrije Universiteit Medical Centre Amsterdam, Prof. dr. C.B. Lambalk, ; Maxima Medical Centre Veldhoven, dr. J.W.M. Maas, ; University Medical Centre Groningen, dr. A. Cantineau, ; Erasmus Medical Centre Rotterdam, Prof. dr. J.S.E. Laven, ; Sint Franciscus Gasthuis, dr. M. van Hooff, ; Onze Lieve Vrouwe Gasthuis Amsterdam, dr. E. Moll, ; Amphia Hospital, Dr. J. Visser, ; Fertility clinics Twente, dr. M. Verberg, ; Diakonessenhuis Utrecht, dr. P.A. Manger, ; Catharina Hospital Eindhoven, dr. M.M.E. van Rumste, ; Deventer Hospital, Ms. L.F. van der Voet, ; Maasstad Hospital, dr. R.H.F. van Oppenraaij, ; Sint Elisabeth-Twee Steden Hospital, dr. J. Smeenk, ; Gelre Hospital Apeldoorn, Ms. M.A.F. Traas, ; Ter Gooi Hospital, dr. C.H. de Koning, ; Reinier de Graaf Gasthuis, Dr. J.C. Boxmeer, ; Groene Hart Hospital Gouda, dr. C.A.H. Janssen, ; Meander Hospital, Mr. E.A. Brinkhuis, ; Noordwest Ziekenhuisgroep, locatie Gemini, Dr. E.R. Groenewoud ; St. Antonius Hospital, dr. J. van Disseldorp, ; Wilhelmina Hospital Assen, Mr. M.W. Glas, ; Medisch Centrum Kinderwens, Dr. A.M. van Heusden, ; Albert Schweitzer Hospital, Ms. M.L. Bandell, ; Leiden University Medical Centre, Ms. N.F. Klijn, ; Haga Hospital, Dr. Q. Pieterse, ; Haaglanden Medical Centre, Ms. S. van der Meer,

Study question: Does mid-luteal endometrial scratching prior to the 2nd stimulation cycle increase live birth rates (LBR) in women with one failed IVF/ICSI cycle?

Summary answer: During follow-up, non-significant 4.0-4.7% increases in LBRs were observed for women that underwent scratching. These differences should be further explored as to their clinical significance.

What is known already: Previous trials on endometrial scratching have been reported to have methodological limitations and high risk of bias making it unclear whether this procedure improves pregnancy rates. Even the recent large PIP trial suffers from the fact that a very heterogeneous study population was included and the timing of scratching was not standardized. Moreover, previous trials only reported the results of a single transfer following randomization. An RCT in a more homogeneous population which standardizes timing and method of scratching, and with a follow-up of 12 months may provide the best information on the effectiveness of scratching.

Study design, size, duration: A multicenter, non-blinded, randomized controlled trial was conducted between January 2016 and July 2018 in the Netherlands. Women were allocated 1:1 to endometrial scratching or no intervention, using a web-based system that ensured allocation concealment. Using an expected difference in LBR after the IVF/ICSI cycle following randomization of 9% (39% vs. 30%, respectively), the sample size was set at 900 participants (80% power and two-sided alpha of 0.05).

Participants/materials, setting, methods: Participants with a failed first IVF/ICSI cycle (≥1 embryo transfer) were eligible. Endometrial scratching was performed using an endometrial biopsy catheter in the mid-luteal phase prior to ovarian stimulation. The primary outcome was LBR from the fresh embryo transfer post-randomization; secondary outcomes included cumulative pregnancy outcomes up to ongoing pregnancy (OP) (achieved within 12 months after randomization) leading to LB. This abstract reports the complete case results for the primary and secondary 12-month follow-up outcomes.

Main results and the role of chance: In total, 936 women were included (468 scratch/468 control). Baseline and infertility characteristics were comparable between the groups. The participation rate was 89% (936/1054 eligibles). A conservative approach was used, in which participants who were lost-to-follow-up were assumed not to have conceived.

The LBR after the post-randomization treatment (i.e. 2nd cycle) was 22.6% in the scratch and 18.6% in the control group (RR 1.21 [95%CI 0.94-1.56]) with a corresponding absolute risk difference (ARD) of 4.0% [95%CI -1.2% to +9.2%]. For the 12-month follow-up, the cumulative LBR was 42.1% vs. 37.4% (RR 1.13 [95%CI 0.96-1.32]) with a corresponding ARD of 4.7% [95%CI -1.7% to +11.1%].

Important strengths include the fairly homogeneous study population, standardized scratching method, 12-month follow-up period, large sample size and high participation rate. The 12-month follow-up period is unique and offers the possibility to study longer-term effects of endometrial scratching. The high participation rate is probably due to the fact that endometrial scratching is not offered as part of clinical care in the Netherlands. This decreases the risk for selection bias and improves the inference for the effect of endometrial scratching for daily practice.

Limitations, reasons for caution: This analysis was based on complete data from 97-99% of the participants, which means the final results (available March 2020) may differ slightly. Despite this high percentage of complete data, complete case analysis reduces precision and may introduce bias. Therefore, in the final dataset residual missing data will be imputed.

Wider implications of the findings: Twelve-month follow-up results showed that a non-significant LBR improvement of 4.0% after the post-randomization treatment in the scratch group is maintained, resulting in a non-significant 4.7% improvement after 12 months. Further analyses in this dataset and an international individual participant data-analysis (IPD) will focus on understanding these findings.

Trial registration number: This trial was prospectively registered in the Dutch Trial Register (Nederlands Trial Register) under number 'NL5193' (old number: 'NTR 5342')

O-077 Ovarian stimulation alters the cervical microbiota

S. Mackens[1], S. Vieira-Silva[2], S. Santos-Ribeiro[3], J. Centelles-Lodeiro[2], A. Racca[1], G. Falony[2], R. Tito[2], H. Tournaye[1], J. Raes[2], C. Blockeel[1]

[1]UZ Brussel, Centre for Reproductive Medicine, Brussel, Belgium ;
[2]Vlaams Instituut voor Biotechnologie VIB - KU Leuven, Center for Microbiology, Leuven, Belgium ;
[3]IVI RMA Lisbon, Reproductive Medicine, Lisbon, Portugal

Study question: Does ovarian stimulation (OS) have an impact on cervical microbiota composition and diversity?
Summary answer: OS significantly influences the cervical microbiota composition and increases its diversity.
What is known already: Disturbances of the female genital microbiota are associated with female sexual health complications such as an increased risk for sexually transmitted infections and with obstetrical complications such as preterm delivery. Recently, it has been suggested that women undergoing IVF/ICSI are particularly prone to genital dysbiosis and that an abnormal microbiota composition and an increased species diversity might affect post-treatment pregnancy rates. However, before introducing reproductive tract microbiota analyses in clinical practice to predict IVF/ICSI outcome, confounders need to be investigated more thorough. For example, the impact of OS remains to be elucidated.
Study design, size, duration: This analysis was part of a prospective observational cohort study investigating the potential effect of the female reproductive tract microbiota on IVF/ICSI outcomes and included 106 women each providing two samples between 2016 and 2019. Samples from the cervical microbiota were collected by swabbing at two different timepoints: prior to OS (baseline) and at the moment of oocyte retrieval (post-OS).
Participants/materials, setting, methods: Caucasian women, aged <40y, planned for a first or second IVF/ICSI cycle performing OS in an antagonist protocol followed by fresh single blastocyst transfer were included. Cervical microbiota samples were retrieved by swabbing, flash-frozen in liquid nitrogen and stored at -80°C. Microbiota profiles were obtained by amplicon sequencing (16SrRNA V4) using the gold-standard DADA2 pipeline. Correlations to microbiota profiles were performed by constrained principal coordinates analysis at genus level (cPCoA with Aitchinson distance).
Main results and the role of chance: OS led to a significant shift in cervical microbiota (n=106x2, paired cPCoA, R^2=0.006, p=0.016). Also, microbial diversity significantly increased during OS (n=106x2, paired t-test, t-ratio=4.80, p=5.4E-6). We evaluated whether the menstrual cycle phase at the moment of baseline sampling confounded this association between OS and the shift in diversity and observed that, in our dataset, this was not correlated (Kruskal-Wallis, p=0.72). The cervical microbiota profiles were typed into 4 different community-types (CTs), two of them having the characteristic dysbiotic high-species diversity. Focusing on 72 patients having achieved live birth (n=39) versus not having reached a +hCG (n=33) after a fresh embryo transfer, CTs were not significantly associated to outcome at both timepoints (Fisher's test, baseline p=0.63, post-OS p=0.27). However, CTs shifted significantly from baseline to post-OS (n=144, Fisher's test, p=0.03). In cervical microbial diversity, also no significant difference was observed between the two outcomes (live birth vs not pregnant), at baseline (n=72, Kruskal-Wallis, ES=0.16, p=0.18), nor after OS (n=72, Kruskal-Wallis, ES=0.13, p=0.28). However, an increase in diversity was confirmed for both outcome groups, with this increase being slightly higher for the negative outcome (n=33x2, paired t-test, t-Ratio=3.50, p=0.001) than for the live birth outcome (n=39x2, paired t-test, t-Ratio=3.11, p=0.004).
Limitations, reasons for caution: Human-associated microbial communities have a high inter-person variability and even with a reasonably scaled study of 106 women, statistical significance is difficult to reach. Caution is warranted as non-significant associations do not prove non-correlations.
Wider implications of the findings: Interest has increased in predicting IVF/ICSI outcome using reproductive tract microbiota analysis. The impact of potential confounders however needs to be assessed first. We found OS to be a significant driver of microbial compositional and diversity variation, labelling it as a potential confounder that deserves more attention in future research.
Trial registration number: NCT03105453

O-078 Uterine peristalsis during implantation period; experience of 4,800 patients with 3 or more failure of embryo transfers

H. Matsubayashi[1], K. Kitaya[1], K. Yamaguchi[1], Y. Ohara[2], M. Doshida[2], T. Takeuchi[2], T. Ishikawa[2]

[1]Reproduction Clinic Osaka, Obstetrics and Gynecology, Osaka, Japan ;
[2]Reproduction Clinic Tokyo, Obstetrics and Gynecology, Tokyo, Japan

Study question: Is uterine peristalsis frequently observed in patients with recurrent implantation failure?
Summary answer: Uterine peristalsis was frequently (47.2%) observed in patients with recurrent implantation failure mostly in the whole uterine cavity with "lower→upper→lower" direction.
What is known already: Uterine peristalsis caused by uterine contraction is thought to be one of the risk factor for implantation failure, because the uterus is quiescent at the time of implantation period.Previous studies suggested more than 2 or 3 waves/min may be a threshold for implantation failure. Although those reports focused on frequency and direction of the uterine contraction, there were no reports regarding intensity and location of the uterine contraction. Therefore, we investigated intensity and location as well as frequency and direction of the uterine contraction in the largest number of patients with recurrent failure of embryo transfers.
Study design, size, duration: Transvaginal ultrasonography scans of uterine peristalsis were performed at the mid luteal phase in 4,800 patients with 3 or more failure of embryo transfers in two clinics between 2013 and 2019. The transvaginal probe (6 to 10 MHz) was introduced into the vagina as gently as possible to avoid stimulating the uterine cervix. After scanning mid-sagittal plane of the uterus, the probe was fixed as steady as possible while 3 min, video was recorded simultaneously.
Participants/materials, setting, methods: The video images were analyzed at 10 time the normal speed using Quick Time Player by a single observer. Frequency, intensity, location and direction of the uterine contractile activity were recorded and evaluated. Intensity was divided into 3 categories; movement with the whole endometrium (strong), with the middle and the surface of the endometrium (medium), and just the surface of the endometrium (weak). Direction was complicated with many patterns (e.g., lower→upper→lower).
Main results and the role of chance: Of 4,800 patients (average age, 37.5), 2,534 (52.8%) did not show any uterine peristalsis, 2,266 (47.2%) had uterine peristalsis. In the peristalsis group, frequency was 57.1% for 1 to 3 (times/3 min), 28.6% for 4 to 6, 10.7% for 7 to 9, and 3.7% for 10 or more. Intensity was almost equal among 3 categories (strong 30.4%, medium 38.8%, weak 30.8%). Most uterine peristalsis was observed in the whole uterine cavity (82.8%), whereas those in the upper, middle and lower part of the uterus were 8.7%, 1.2% and 7.3%, respectively. In terms of direction, about half (52.1%) of uterine peristalsis was observed as "lower→upper→lower", followed by "upper→lower→upper" (15.8%), "lower→upper" (13.8%), "upper→lower" (13.3%), and unfocused (4.9%). Pregnancy outcome of patients (N=29) who had strong uterine peristalsis with 10 or more was retrospectively evaluated after taking piperidolate hydrochloride (150 mg/day). Patients with live birth or ongoing pregnancy with 28 weeks or more were 13 (44.8%), those with biochemical pregnancy or miscarriage were 7 (24.1%), and those without pregnancy were 9 (31.0%).
Limitations, reasons for caution: Since this is a retrospective observational study, a prospective randomized study is necessary to determine the cutoff value that should be treated for uterine peristalsis in patients with recurrent implantation failure.
Wider implications of the findings: These data suggest that uterine peristalsis was frequently (47.2%) observed in patients with recurrent implantation failure, mostly in the whole uterine cavity with direction as "lower→upper→lower". However, we have to determine the cutoff value that should be treated. Further studies will be required.
Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS
SESSION 20: REPRODUCTIVE (EPI)GENETICS 1
06 July 2020 Parallel 4 15:15 - 16:30

O-079 Chromosome errors in human eggs shape natural fertility

J. Gruhn[1], A. Zielinska[2], V. Shukla[1], R. Blanshard[3,4], A. Capalbo[5,6], D. Nikiforov[7,8], A.C.H. Chan[1], L. Newnham[4], I. Vogel[1], R. McCoy[9], G. Hartshorne[10], K. Lukaszuk[11,12,13], C.Y. Andersen[7], M. Schuh[2], E. Hoffmann[1,4]

[1]Univeristy of Copenhagen, Center for Chromosome Stability, København N, Denmark ;
[2]Max Planck Institute for Biophysical Chemistry, Department of Meiosis, Göttingen, Germany ;
[3]Illumina Inc., Clinical Genomics Group, Fulbourn, United Kingdom ;
[4]University of Sussex, Genome Damage and Stability Centre- School of Life Sciences, Sussex, United Kingdom ;
[5]University of Rome, Dipartimento di Scienze Anatomiche- Istologiche- Medico-Legali e dell'Apparato Locomotore- Sezione Istologia ed Embriologia Medica, Rome, Italy ;
[6]IGENOMIX, n/a, Marostica, Italy ;
[7]Univeristy of Copenhagen, Laboratory of Reproductive Biology- The Juliane Marie Centre for Women- Children and Reproduction- Copenhagen University Hospital and Faculty of Health and Medical Sciences, København N, Denmark ;
[8]Università degli studi di Teramo, Unit of Basic and Applied Biosciences, Teramo, Italy ;
[9]Johns Hopkins University, Department of Biology, Baltimore, U.S.A. ;
[10]University of Warwick, Warwick Medical School- University of Warwick Centre for Reproductive Medicine- University Hospital Coventry, Warwick, United Kingdom ;
[11]INVICTA, Fertility and Reproductive Center, Gdansk, Poland ;
[12]Medical University of Warsaw, Department of Gynecological Endocrinology, Warsaw, Poland ;
[13]Medical University of Gdansk, Department of Obstetrics and Gynecological Nursing- Faculty of Health Sciences, Gdansk, Poland

Study question: How do the rate and causes of aneuploidy within human oocytes change throughout the reproductive lifespan?
Summary answer: Distinct types of chromosomal errors drive aneuploidy in human oocytes in an age-dependent manner, thereby regulating reproductive capacity in young girls and older women.
What is known already: Aneuploidy affects an exceptionally high number of human conceptions, resulting in congenital disorders or pregnancy loss. Most focus has been on women of advanced maternal age by studying oocytes donated during fertility treatment. What is less appreciated is that aneuploidy rates in clinically-recognized pregnancies are also elevated in teenagers that are by default excluded from IVF based studies. We currently do not understand whether the elevation in teenagers is due to errors in oocytes, sperm, or embryonic mitoses. This study provides insight into the mechanisms behind the incidence of aneuploidy across the entire human reproductive lifespan.
Study design, size, duration: This is a multicenter study based on >3000 oocytes collected from 268 girls and women from two independent cohorts. Small antral follicles (cohort 1) were collected directly from ovarian tissue of unstimulated girls and women undergoing ovarian cortex cryopreservation prior to chemotherapy (9.1-38.8 years). Mature and immature oocytes (cohort 2) were collected from gonadotrophin-stimulated women being treated in *in-vitro* fertilization (IVF) clinics (20-43 years).
Participants/materials, setting, methods: Oocytes were obtained from women covering a majority of the reproductive lifespan (9-43 years) by *in-vitro* maturation of small antral follicles (cohort 1) or immature oocytes (cohort 2) to the mature meiosis II (MII) stage. The oocyte and polar body were isolated and underwent whole genome amplification, followed by both Next Generation Sequencing (NGS) and SNP array analysis. Chromosome segregation errors were identified by NGS based chromosome content.
Main results and the role of chance: The rate of chromosomal abnormalities in oocytes follows a U-shaped curve, suggesting that aneuploidies at both young and advanced ages are female in origin. Unexpectedly, specific segregation errors showed different age-dependencies, therefore providing a quantitative explanation for the U shape. Increased aneuploidy in young girls and women (<20) was preferentially associated with whole-chromosome non-disjunction (MI NDJ) events. Whereas women of advancing maternal age (≥33) showed centromeric or more extensive cohesion loss through premature separation of sister chromatids (PSSC) and reverse segregation (RS) events, respectively. These results were validated directly in human oocytes of two independent patient cohorts, as well as a third cohort of pre-implantation embryos.
Limitations, reasons for caution: None of our samples represent a completely 'normal' setting, due to either in-vitro maturation or hormone stimulation. However, the U-curve was reproducible in three independent datasets, including clinically relevant mature MII oocytes from gonadotrophin-stimulated women.
Wider implications of the findings: Our findings suggest that age-dependent chromosomal errors originating directly in oocytes may shape the curve of natural fertility in humans.
Trial registration number: not applicable

O-080 preimplantation genetic testing for aneuploidy: evaluation of age, indication and embryological parameters for detection of different types of aneuploidy

S. Ecemis[1], K. Boynukalin[2], M. Gultomruk[3], E. Turgut[4], M. Bahceci[4]

[1]Bahceci IVF Ankara Centre, Gynecology- Obstetrics and Infertility, Ankara, Turkey ;
[2]Bahceci IVF Fulya Centre, Gynecology- Obstetrics and Infertility, Istanbul, Turkey ;
[3]Bahceci IVF Fulya Centre, Reproductive Genetics, Istanbul, Turkey ;
[4]Bahceci IVF Fulya Centre, Gynecology- Obstetrics and Infertility, Istanbul, Turkey

Study question: Do any patient and embryo characteristics allow us to predict aneuploidy or the type of aneuploidy (single, double, segmental or complex)?
Summary answer: Female age was the sole significant factor affecting the ploidy status of the embryo. Clinical and embryological features did not reach to statistical significance.
What is known already: The correlation of morphology and morphokinetic parameters of embryos with embryo ploidy status have been evaluated in many studies. Still the evidence is scarce and correlation statement is moderate. The embryos those were generated from the same cohort of a patient might be affected by treatment related external factors in a same manner. The type of aneuploidy such as single (trisomy, monosomy, segmental), double (monosomy/monosomy, monosomy/trisomy, trisomy/trisomy, segmental/segmental, segmental/aneuploidy) or complex has not been studied in detail.
Study design, size, duration: This is a single center retrospective observational study performed between January 2016 and January 2019. The study includes the data analysis of 1793 blastocysts with conclusive comprehensive chromosome screening (CCS) by Next Generation Sequencing (NGS) of trophectoderm (TE) biopsies obtained from 591 patients. Female age under 35 years included in the study.
Participants/materials, setting, methods: PGT-A was offered to infertile couples with recurrent implantation failure(RIF) and repetitive miscarriages (RM), previous history of aneuploidy, severe male factor, and for patient anxiety. TE biopsy was performed on day 5 or day 6 to hatching blastocysts and Gardner classification was used as morphological grading. Correlations between patient, treatment and embryological parameters and euploidy status were tested in Generalized Linear Mixed Model using Binomial Distribution as Probability distribution.
Main results and the role of chance: The median female age of this patient population was 32 (30-33). Of the 1793 biopsied blastocysts, 800 were aneuploidy, 963 were euploid. Regarding the day of biopsy, the euploidy rate were significantly different between day 5 and day 6 biopsied embryos (830/1304(63.7%) vs 281/489(57.5%), p=0.016 respectively). Aneuploid blastocysts showed poor quality ICM [15.4%(105/682) vs 9.1%(101/1111); p<0.001] and TE [75%(512/682) vs 56.3%(626/1111); p<0.001]. The frequency of different abnormal chromosomal patterns as single, complex aneuploid and chaotic patterns were not related to the day of biopsy (p = 0.166) as well as to the ICM (p=0.12) and TE (p=0.89) score. We observed an increasing probability for aneuploidy with female age of 3% per year (p<0.015) even though the patient population was <35 years of age. No statistically significant relation was found between aneuploidy and the other clinical and laboratory findings.
Limitations, reasons for caution: The study is limited by its retrospective nature. Higher sample size or a prospective design within the morphokinetic parameters could be used in future studies to corroborate the current findings.

Wider implications of the findings: This study reports that conventional morphologic parameters are insufficient for predicting the euploid embryo and the type of aneuploidy. Also the patient characteristics other than female age are not predictive. infertility diagnosis or PGT-A indications does not characterize the ploidy status. Future research to identify non-invasive biomarkers should be performed.
Trial registration number: none

O-081 New evidence on mosaic developmental potential: multicentric study of 822 mosaic embryos diagnosed by preimplantation genetic testing with trophectoderm biopsy

F. Spinella[1], E. Greco[2], A. Victor[3], M.G. Minasi[2], F. Barnes[3], C. Zouves[3], J. Grifo[4], E.H. Cheng[5], S. Munnè[6], A. Biricik[1], M. Surdo[1], M. Baldi[1], A. Ruberti[2], F. Fiorentino[1], M. Viotti[3]

[1]Genoma Group srl, Molecular Genetics Laboratories, Rome, Italy ;
[2]European Hospital, Centre For Reproductive Medicine, Rome, Italy ;
[3]Zouves Fertility Center, Fertility Center, Foster City, U.S.A. ;
[4]New York University, Langone Fertility Center, New York, U.S.A. ;
[5]Lee Women's Hospital, Lee Women's Hospital, Taichung, Taiwan R.O.C. ;
[6]Cooper Genomics, Cooper Genomics, Livingston, U.S.A.

Study question: Is the clinical outcome of mosaic embryos influenced by chromosomal constitution?
Summary answer: Reproductive potential of mosaic embryos is affected by the complexity of and the number of aneuploidy cells present in trophectoderm (TE) biopsy.
What is known already: Chromosomal mosaic embryos are characterized by the presence of chromosomally different cell lines within the same embryo. While the transfer of these embryos is now offered as an option for women who undergo in vitro fertilization (IVF), several concerns remain. For instance, the limited data on pregnancy outcome and the possibility that intra-biopsy mosaicism in the TE is a poor predictor of the ploidy status of the ICM. Therefore, some argue that mosaicism should be not reported until a clear classification of such embryos in relation with their reproductive potential has been defined.
Study design, size, duration: We collected the clinical outcomes of 822 mosaic embryos transferred in women underwent IVF between May 2016-May 2019. All embryos were cultured to blastocyst stage; trophectoderm (TE) biopsy was performed on Day-5 of development or Day6/7 for slow growing embryos. The clinical outcome obtained after transfer of mosaic embryos with different chromosomal constitution was compared with each other and with that obtained from a control group of 3781 euploid blastocysts.
Participants/materials, setting, methods: Preimplantation genetic testing (PGT) was performed using high resolution next generation sequencing (NGS) methodology. TE biopsies were classified as mosaic if they had 20%-80% abnormal cells. For statistical analysis mosaic embryos were divided in groups based on mosaic levels and chromosomal constitution detected in TE: single mosaic aneuploidy (monosomy/trisomy; SM), double mosaic chromosomes (monosomy/trisomy or combination, DM), complex mosaic aneuploidy (>2 different aneuploidies; CM) and mosaic segmental aneuploidy (single and double deletion/insertion >5Mb, MS).
Main results and the role of chance: The embryos were plotted in 10% increments, representing a progressive increase in the proportion of aneuploid cells in the TE, and linear regression showed a statistically significant decline in rates of implantation and ongoing pregnancy/birth (regression function with respective slopes -0.42 and -0.55, P=0.0381 and 0.0099). Regarding chromosomal constitution, MS had the best outcomes, followed by the group with one affected chromosome, followed by the group with two affected chromosomes, followed by the complex group (implantation P<0.0001, ongoing pregnancy/birth P<0.0001). MS showed a significantly poorer clinical outcomes compared to the euploid control group (implantation 51.3% vs 61.1%, P=0.0004; ongoing pregnancy/birth 42.6% vs 52.7%, P=0.0003).
Limitations, reasons for caution: Additional clinical data must be obtained to evaluate the contribution of each different chromosome before this approach can be evaluated as an additional tool to choose mosaic embryos for transfer.
Wider implications of the findings: The study provides the largest dataset of transferred mosaic embryo outcomes reported to date. This compiled analysis conclusively shows that embryos with different pattern of chromosomal mosaicism have a distinct set of clinical outcomes. This findings should be considered for genetic counseling.
Trial registration number: None

O-082 The application of more stringent parameters for mosaic classification in blastocysts-stage primplantation genetic testing for aneuploidies reduces false positive mosaic rates without comprising true detection

L. Girardi[1], C. Patassini[1], M. Fabiani[1], S. Caroselli[1], M. Serdarogullari[2], O. Coban[2], N. Findikli[3], K. Boynukalin[4], M. Bahceci[4], R. Chopra[5], R. Navarro[6], M. Poli[1], C. Simón[7,8,9,10], C. Rubio LLuesa[11], A. Capalbo[1,7]

[1]Igenomix, Reproductive Genetics, Marostica, Italy ;
[2]British Cyprus IVF Hospital, Embryology Laboratory, Nicosia, Cyprus ;
[3]Bahceci Fulya IVF Centre, IVF Laboratory, Istanbul, Turkey ;
[4]Bahceci Fulya IVF Centre, Infertility Clinic, Istanbul, Turkey ;
[5]Igenomix FZ LLC, Dubai Health Care City, Dubai, United Arab Emirates ;
[6]Igenomix, Igenomix R&D, Valencia, Spain ;
[7]Igenomix Foundation, Reproductive genetics, Valencia, Spain ;
[8]Baylor College of Medicine, Department of Obstetrics and Gynecology, Houston-TX, U.S.A. ;
[9]Harvard University, Harvard School of Medicine- Department of Obstetrics and Gynecology, Boston, U.S.A. ;
[10]Valencia University- and INCLIVA, Department of Obstetrics and Gynecology, Valencia, Spain ;
[11]Igenomix, PGS Research, Valencia, Spain

Study question: How do mosaic diagnostic thresholds setting affect the accuracy of Next Generation Sequencing (NGS)-based preimplantation genetic testing for aneuploidies (PGT-A)?
Summary answer: When single trophectoderm biopsy is tested, wide mosaicism thresholds (i.e.,20-80%) increase false positive calls compared to more stringent ones (i.e.,30-70%) without improving true detection rate.
What is known already: Highly sensitive NGS-based technologies for PGT-A allows precise identification of intermediate chromosome copy number alterations potentially associated to chromosomal mosaicism in trophectoderm biopsies. Nevertheless, differences in technical validation procedures and in detection thresholds employed for diagnostic calls could lead to incorrect classification of normal and abnormal embryos into the mosaic category. Overcalling mosaicism in trophectoderm biopsies lowers PGT-A accuracy, ultimately affecting patients treatment outcome from both a clinical and a psychological standpoint. In this study, we evaluated diagnostic predictivity of different mosaicism classification criteria by employing blinded analysis of chromosome copy number values (CNV) in multifocal blastocyst biopsies.
Study design, size, duration: The accuracy of different mosaicism diagnostic cut-offs was assessed comparing chromosomal CNV in intra-blastocysts multifocal biopsies. Enrolled embryos were donated for research between January and December 2019. The Institutional Review Board at the Near East University approved the study (project: YDUI20191170-849). Embryos showing euploid/aneuploid mosaicism in their clinical trophectoderm (TE) biopsy (n=36) and euploid embryos (n=23) were disaggregated into 5 portions: the inner cell mass (ICM) and 4 TE biopsies. Overall, 295 specimens were analysed.
Participants/materials, setting, methods: Fifty-nine donated blastocysts were warmed, allowed to re-expand and disaggregated in TE biopsies and ICM. PGT-A analysis was performed using Ion ReproSeq PGS kit and Ion S5 sequencer (ThermoFisher). Sequencing data were blindly analysed with Ion-Reporter software. Intra-blastocyst comparison of raw NGS data was performed employing different thresholds commonly used for mosaicism detection. CNV for each chromosome were reported as mosaic, according to 30-70% and 20-80% criteria. Categorical variables were compared using Fisher's exact test.
Main results and the role of chance: To minimize the impact of technical over biological variation, intermediate CNV were classified as confirmed mosaic according to the following criteria: 1) detection of the same mosaic alteration in at least 3 biopsies or in 1 additional biopsy over the 50% threshold, or 2) detection of a reciprocal mosaic pattern involving the same chromosome. If the same alteration was uniformly detected (>50%) in all biopsies, the embryo was classified as uniform aneuploid. When the high mosaicism threshold was considered (50-70%), the aneuploidy finding, mosaic or uniform, was confirmed in 82.5% of patterns (14/17; 95%CI=54.6-96.2). In particular, 35.3% of cases (6/17; 95%CI=3.0-16.8) were uniform aneuploid. For the low mosaicism category, 30-50%, putative mosaicism was confirmed in only 5.3% of the cases (3/57; 95%CI=1.10-14.62). When 20-50% threshold was applied, a significantly higher

number of false mosaic alterations were observed, and the confirmation rate dropped to 1.8% (n=3/168;95%CI=0.37-5.13; P<0.001). In particular, the inclusion of very-low mosaicism (20-30%) results only added false positive results but no true mosaic case (66.1%;n=111/168;95%CI=58.38-73.19;P<0.001). In the euploid embryos group, none of the results obtained in the 39 cases of 20-30% and in the 30-50% range were confirmed.

Limitations, reasons for caution: The study involved only blastocysts initially diagnosed as euploid or mosaic. Uniform aneuploid embryos were not evaluated at this stage. This approach involved the analysis of mosaicism thresholds at the embryo level and future studies will need to evaluate these criteria in relation to clinical predictive values following embryo transfers.

Wider implications of the findings: Based on an embryo re-biopsy model, single TE biopsy results showing low mosaicism, particularly the very-low range (20%-30%) shouldn't be considered as mosaicism diagnoses. The application of the lower stringency threshold for mosaic classification (i.e.,20%) leads to misclassification of embryos, increasing false positive calls and lowering accuracy of PGT-A analysis.

Trial registration number: N/A

O-083 Incidence of mosaic embryos in translocation carriers: Are translocation carriers more predisposed to generate mosaic embryos?

M. Cetinkaya[1], M.A. Tufekci[1], C. Cinar Yapan[1], S. Kahraman[1]

[1]Istanbul Memorial Hospital, Assisted Reproductive Technologies and Reproductive Genetics Center, Istanbul, Turkey

Study question: Is the mosaicism ratio influenced by the sex of the translocation carrier and the type of translocation in preimplantation genetic testing for structural rearrangements (PGT-SR)?

Summary answer: The mosaicism ratio is significantly higher in Robertsonian translocation carriers (RobTC) (7x) and in male translocation carriers (3x).

What is known already: Embryonic mosaicism is defined as the presence of karyotypically distinct cell lines within an embryo and can be detected with Next Generation Sequencing (NGS) at a 20-80% rate. High incidence of mosaicism has been reported in preimplantation embryos, with the blastocyst mosaicism being between 4-24% (Harton et al., 2017). Although mosaic embryos have a chance to implant (Munne et al., 2017; Spinella et al., 2018) their pregnancy rate is lower, with an increased miscarriage rate. However, female and male translocation carriers, whether Robertsonian or reciprocal, have not been yet analyzed regarding the incidence of generating mosaic embryos.

Study design, size, duration: This retrospective study was based on 143 PGT-SR cycles initiated between January 2017 and September 2019. A total number of 573 blastocysts were tested for PGT-SR by ReproSeq on Ion Torrent S5 (Thermo Fisher Scientific) following trophectoderm biopsy.

Participants/materials, setting, methods: The number of blastocysts tested from female and male translocation carriers were 284 and 289, respectively. The number of blastocysts tested from reciprocal translocation carriers was 471 whereas 102 were from RobTC. The mean female age in all groups was similar and statistically non-significant (32.8 and 31.2 for female and male and 33.3 and 31.7 for Robertsonian and reciprocal translocation carriers). Chi-square test was used for categorical group comparisons.

Main results and the role of chance: The overall mosaicism rate for PGT-A was 11% (8239 trophectoderm biopsies). The euploid/ balanced translocation rate of Robertsonian and reciprocal translocation carriers was 32.4% and 20.4%, respectively (p=0.008). The frequency of mosaic embryos generated by RobTC was seven times higher than carriers of reciprocal translocations (21.6% and 3.2%; p<0.0001).

Also, male translocation carriers in general were three times more prone to have mosaic embryos than female translocation carriers (9.3% and 3.5%; p=0.046). The euploid/ balanced translocation rate was identical for male and female translocation carriers (22.5%).

When the subgroups were analyzed, female RobTC were found to generate nearly seven times more mosaic embryos than female reciprocal translocation carriers (15.4% and 2.3%; p<0.001). Moreover, male RobTC engendered nearly six times more mosaic embryos than male reciprocal translocation carriers (23.7% and 4.2%; p<0.0001).

One possible mechanism to explain the increased mosaicism in RobTC may be the involvement of the centromere in balanced translocation carrier embryos. This involvement may lead to errors in mitotic divisions during embryo development. Embryos of RobTC have been shown to exhibit a mitotic interchromosomal effect that enhances genetic instability during early development (Alfarawati et al., 2012). Also, 30% of RobTC were diagnosed as having a severe male factor.

Limitations, reasons for caution: The limitation of our study was being a retrospective analysis. A prospective follow-up study with a larger sample size is needed to verify the outcome of this abstract.

Wider implications of the findings: As the possibility of finding an euploid or balanced carrier embryo is relatively lower for translocation carriers, the transfer of a mosaic embryo may be a realistic option. Higher incidence of mosaicism in Robertsonian/ male translocation carriers may provide an insight to understanding the mechanism of mosaicism in human embryos.

Trial registration number: -

SELECTED ORAL COMMUNICATIONS

SESSION 21: IMPACT OF NEW TECHNOLOGIES ON HUMAN REPRODUCTION

06 July 2020 Parallel 5 15:15 - 16:30

O-084 A construct of mesenchymal stem cell-derived exosomes/ collagen scaffold promotes endometrium regeneration and fertility restoration through macrophage immunomodulation

L. Xin[1]

[1]Zhejiang University, School of Medicine, Hangzhou, China

Study question: Can a construct of mesenchymal stem cell-derived exosomes/collagen scaffold provide a new treatment for Asherman's syndrome?

Summary answer: A construct of mesenchymal stem cell-derived exosomes/ collagen scaffold promotes endometrium regeneration and fertility restoration through macrophage immunomodulation

What is known already: Endometrial traumas caused by recurrent curettage, caesarean section and myomectomy always result in intrauterine adhesions (IUAs) and infertility. Umbilical cord-derived mesenchymal stem cell (MSC)-based therapies have shown some promise achievements in the treatment of IUAs for their easy collection, high proliferation and low immunogenicity. However, the potential tumorigenicity, low infusion and low retention of MSCs are still controversial and the clinical application of MSCs is limited. In contrast, MSC-derived exosomes exhibit a similar function to their source cells and are expected to overcome these limitations.

Study design, size, duration: A total of approximately 200 SD rats were involved and received different treatments. Uteri were examined at 1,3,7, 30 and 60 days after surgery. We evaluated the effects of CS/Exos on the regeneration of damaged endometrium and the restoration of fertility, as well as exploring the healing mechanism from the aspect of macrophage immunomodulation by functional miRNAs *in vivo* and *in vitro*.

Participants/materials, setting, methods: we designed a construct of exosomes and a collagen scaffold (CS/Exos) for endometrium regeneration, and investigated the regeneration mechanism through macrophage immunomodulation. PCR,ELISA, IF, IHC, HE staining, Masson's staining and RNA-seq were involved.

Main results and the role of chance: The CS/Exos transplantation potently induced (i) endometrium regeneration, (ii) collagen remodeling, (iii) increased the expression of the estrogen receptor a/progesterone receptor, and (iv) restored fertility. Mechanistically, CS/Exos facilitated $CD163^+$ M2 macrophage polarization, reduced inflammation, and increased anti-inflammatory responses *in vivo* and *in vitro*. By RNA-seq, miRNAs enriched in exosomes were the main mediator for exosomes-induced macrophage polarization. Overall, we demonstrated that CS/Exos treatment facilitated endometrium regeneration and fertility restoration by immunomodulatory functions of miRNAs.

Limitations, reasons for caution: The results of this research are inadequate to apply directly in human because human uterine and rat uterus differ in structure and function. Further large animal experiments, such as monkeys, are needed.
Wider implications of the findings: Our research highlights the therapeutic prospects of CS/Exos for the management of IUAs.
Trial registration number: not applicable

O-085 Differentiation of stromal cells into theca cells: understanding theca cell formation in human ovaries

P. Asiabi[1], M.M. Dolmans[1], J. Ambroise[2], A. Camboni[1], C. Amorim[3], Andrade[1]

[1]Pôle de Recherche en Gynécologie- Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain- 1200, Brussels, Belgium ; [2]Centre de Technologies Moléculaires Appliquées- Institut de Recherche Expérimentale et Clinique-, Université Catholique de Louvain- 1200, Brussels, Belgium [3]Université Catholique de Louvain (UCL) -Institut de Recherche Expérimentale et Clinique Laboratoire de Gynécologie - 1200, Brussels, Belgium

Study question: Is there a subpopulation of precursor theca cells (PTCs) in human ovarian cortex or all can ovarian stromal cells differentiate into theca cells (TCs)?
Summary answer: Around 43% of ovarian stromal cells (SCs) can differentiate into TCs, which indicates the presence of PTCs in human ovaries.
What is known already: There are very few studies on the origin of TCs in mammalian ovaries. Progenitor TCs have been described in neonatal mice ovaries, which can differentiate into TCs under the influence of factors from oocytes and granulosa cells. On the other hand, studies in large animal models have reported that SCs isolated from the cortical ovarian layer can also differentiate into TCs with the right stimuli.
Study design, size, duration: After obtaining informed consent, ovarian biopsies were taken from eight menopausal women (53-74 years of age) undergoing laparoscopic surgery for gynecologic disease not related to the ovaries. SCs were isolated and then in vitro cultured either in basic medium (G1) or enriched with growth factors, follicle-stimulating hormone and luteinizing hormone (G2) for 8 days.
Participants/materials, setting, methods: To confirm whether SCs were able to differentiate into TCs, relative mRNA levels for LHCGR, StAR, CYP11A1, CYP17A1, HSD3B1 and 2 were assessed. Immunohistochemistry (IHC) was also performed for their protein detection and a specific marker for theca interna cells (CD13). Finally, we analyzed the ultrastructure of the cells before (D0) and after (D8) in vitro culture, and DHEA and progesterone levels in the medium using transmission electron microscopy and ELISA respectively.
Main results and the role of chance: Quantitative PCR results showed a significant ($p<0.05$) increase in mRNA levels of HSD3B2 in G1 and G2 and CYP17A1 in G1 after 8 days of in vitro culture. IHC results confirmed expression of each enzyme involved in the steroidogenic pathway at the protein stage. However, only G2 exhibited a significant proportion (43%) of CD13-positive cells compared to cells soon after isolation (0%) and in vitro-cultured in basic medium (0%). Ultrastructural analyses showed a distinct difference between the two groups versus D0. Linear trends displayed a significant rise ($q<0.001$) in dehydroepiandrosterone (DHEA) and progesterone concentrations in medium in G2 culture with time. Similarly, G1 exhibited a significant upturn ($q<0.001$) in DHEA, but not progesterone, which remained the same throughout the culture period. Statistical comparisons of progesterone and DHEA levels on day 8 for G1 versus G2 were $q<0.05$ and $q=0.33$ respectively.
Limitations, reasons for caution: While our results indicate the presence of PTCs in the human ovary, as after in vitro culture, 43% of cells express TC genes and proteins and are able to synthesize steroids. It is now necessary to identify specific markers for PTCs to confirm our findings.
Wider implications of the findings: Results from current experiments are a promising step towards understanding TC ontogenesis in the human ovary. Moreover, in vitro-generated human TCs can be used for studies on drug screening, as well as to understand TC-associated pathologies, such as androgen-secreting tumors and polycystic ovary syndrome.
Trial registration number: not applicable

O-086 Embryo development of reconstituted zygotes using haploid androgenotes obtained from ooplast-mediated male genome cloning

A. Petrini[1], P. Xie[1], A. Trout[1], R. Setton[1], Z. Rosenwaks[1], G. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Is it feasible to clone the male genome by utilizing haploid androgenic blastomeres as male gametes to reconstitute diploid zygotes supporting full preimplantation development?
Summary answer: Haploid androgenotes are capable of complementing female pronuclei, allowing reconstitution of diploid zygotes that completed a normal preimplantation development comparable to normally fertilized conceptuses.
What is known already: In cases of severely impaired spermatogenesis in which only a limited number of gametes is available, or in cases of gametal heterozygosity for heritable diseases, male gamete cloning has been sought and attempted. Male genome cloning can be achieved through an ooplast-mediated approach that generates androgenic embryos, which cleave to the 2-, 4-, or 8-cell stage while maintaining the identical genotype as the injected spermatozoa and can function as a male gamete. In addition, the propagation of the male genome allows pre-fertilization genetic testing.
Study design, size, duration: Haploid male embryos were generated and allowed to cleave to duplicate the male genome. Resulting androgenotes were isolated immediately after first cleavage and treated with DNA polymerase inhibitor to pause cell cycle before the S phase. Monopronucleated recipient oocytes were fused with androgenotes to reconstitute diploid embryos. Constructs were maintained in culture up to 96h in a time-lapse system to assess all steps of preimplantation development. Piezo-actuated ICSI was performed on untreated oocytes as control.
Participants/materials, setting, methods: Metaphase II oocytes from B6D2F1 mice were treated with cytochalasin B and enucleated by excising the spindle herniation under OosightÔ visualization. Resulting ooplasts were injected with spermatozoa from the same strain and allowed to reach the 2-cell stage. Resulting androgenotes were exposed to 1.5 mM aphidicolin until reconstitution and fused with monopronucleated recipient oocytes from another cohort using Sendai virus. Cleavage parameters of resulting zygotes were obtained by time-lapse comparing to ICSI conceptuses as control.
Main results and the role of chance: A total of 59 oocytes were all successfully enucleated. The resulting 59 ooplasts were injected with sperm heads; 44 survived (75%), and all developed a single male pronucleus 4-6h post-ICSI. After culturing up to 16h, 42 (95%) constructs cleaved to the 2-cell stage, yielding 84 haploid androgenotes. Parthenogenic activation by calcium ionophore was successful in 93% of the recipient oocytes, confirmed by the extrusion of the second polar body and the appearance of a single female pronucleus. A total of 42 haploid androgenotes were subzonally inserted with Sendai virus and successfully fused with corresponding activated oocytes at a rate of 92.9%. Untreated oocytes were inseminated by piezo-actuated ICSI serving as control, yielding 30 zygotes. The cleavage of reconstructed embryos into the 2-cell stage (89.7%), 4-cell stage (87.2%), morula compaction (84.6%), and blastocyst formation (76.9%) was comparable to control ICSI conceptuses (86.7%, 83.3%, 80.0%, and 80.0%, respectively), with no noticeable morphokinetic differences.
Limitations, reasons for caution: While this approach is successful in a mouse model, its application to human reproduction requires serious consideration. Major concerns include the retention of imprinting of the male genome during the development of the pseudo-blastomere. Other biological hindrances include the role of the human sperm centrosome and heteroplasmy.
Wider implications of the findings: This technique offers the possibility of replicating a male gamete and may improve our understanding of reprogramming immature germ cells as gametes. Male genome cloning may benefit patients with heritable diseases who need pre-fertilization gamete screening or the use of genomic editing to provide healthy or genome-corrected functional pseudo-gametes.
Trial registration number: not applicable

O-087 Use of intraovarian platelet rich plasma does not increase the ovarian reserve markers,ovarian response or IVF outcome in Bologna poor responders.

S. Singh[1], S. Singh[1], N. Ashraf[1], R. Basheer[1], M. Ashraf[1]

[1]Craft Hospital And Research Center, Reproductive Medicine, Thrissur, India

Study question: Does administration of autologous intraovarian platelet rich plasma (PRP) increase the ovarian reserve markers,ovarian response or IVF outcome in Bologna poor ovarian responders (POR)?
Summary answer: There is no benefit of autologous intraovarian PRP in increasing AMH,AFC,ovarian response to ovarian stimulation or IVF outcome in Bologna POR women.
What is known already: Poor ovarian responders are the most difficult subgroup of patient to treat in ART.The challanges in treatment includes high risk of cycle cancellation,low number of oocytes/embryos and very low pregnancy rates.Many interventions have been tried in these women including androgen supplementation,LH supplementation,growth hormone etc,but none has been clearly shown to be beneficial in improving the take home baby rate.PRP has been utilised as a regenrative therapy by many specialities of medicine since it is rich in growth factors and cytokines.It's use in reproductive medicine has been tried in endometrial regeneration,but data in poor responders for improving treatment outcome is lacking.
Study design, size, duration: A prospective observational cohort pilot study was performed over a period of 18 months on 30 POR reproductive aged women diagnosed based on Bologna criteria at craft hospital,Kerala,India.All the women previously had atleast 1 failed IVF at our center and had details of previously performed ovarian reserve testing available within last 3 months.All the couples had detailed counselling about the experimental nature of the procedure and written consent was obtained.Internal ethical committee approval was taken.
Participants/materials, setting, methods: PRP prepared from autologus blood following standard protocols in mid-late follicular phase was injected under ultrasound guidance into the ovarian cortex under conscious sedation, using a 35 cm 17 G needle at 2 sites in each ovary. After a period of 2 months,antral follicle count (AFC) and anti-mullerian hormone (AMH) were reassesed and 2nd IVF was performed by antagonist protocol.The results of post-PRP values of AFC,AMH and IVF outcome were compared to the pre-PRP value.
Main results and the role of chance: A total of 30 POR women (mean age +/- SD: 38.7 +/- 4.6) were included in the study.The ovarian reserve markers and IVF outcome prior to administration of intraovarian PRP (pre-PRP group) was compared to the the parameters obtained after intraovarian PRP (post-PRP group) The pre-PRP and post-PRP group has similar AFC (4.2 +/- 2.6 vs 3.8+/- 1.8; p>0.05) and AMH (0.46 +/- 0.26 vs 0.49 +/- 0.22; p>0.05). When compared to the IVF cycle performed pre-PRP administration,the post-PRP group showed no difference in the number of growing follicles of >/= 14 mm (3.7 +/- 2.2 vs 4.0 +/- 2.4) , number of MII oocytes (3.2 +/- 1.2 vs 3.5 +/-1.7), fertilization rate (2.8 +/- 1.2 vs 2.6 +/- 1.7) and cleavage stage embryos (1.8. +/- 1.1 vs 2.0 +/- 1.4) respectively; p>0.05 for all). In 5 women (16.6%),cycle had to be cancelled due to lack of follicular growth after 5 days of gonadotrophin administration. 3 women (10%) did not have any embryos to be transferred and hence the cycle was cancelled.Embryo transfer was performed in total of 22 women.Clinical pregnancy rate of 13.3 % (4/30) was achieved with a live birth rate of 10% (3/10).
Limitations, reasons for caution: This study has limitation of being observational cohort study with the women serving as their own historical controls. The study also has a small smaple size.The data presented here were analysed 2 months after the administration of PRP.Whether waiting for a longer duration would have changed the outcome is not known.
Wider implications of the findings: Autologus intraovarian PRP administration has not been shown to improve AMH,AFC,ovarian response or IVF outcome in Bologna poor responder women.It's use should be restriced strictly to research settings to prevent women being exposed to this unnecessary intervention till further evidence to the contrary through well designed randomised controlled trials.
Trial registration number: Not applicable

O-088 Bone marrow derived stem cells restore ovarian function and fertility in premature ovarian insufficiency women. Interim report of a randomized trial: mobilization versus ovarian injection

N. Pellicer De Castellvi[1], S. Herraiz[2], M. Romeu[3], S. Martinez[3], A. Buigues[4], I. Gomez-Seguí[5], J. Martinez[6], A. Pellicer[7]

[1]La Fe University Hospital- Joint first authors, Women´s Health Area, Valencia, Spain ;
[2]La Fe University Hospital- Joint first authors, IVI Foundation-Health Research Institute La Fe, Valencia, Spain ;
[3]La Fe University Hospital, Women´s Health Area, Valencia, Spain ;
[4]La Fe University Hospital, IVI Foundation-Health Research Institute La Fe, Valencia, Spain ;
[5]La Fe University Hospital, Hematology Department, Valencia, Spain ;
[6]La Fe University Hospital, Radiology Department, Valencia, Spain ;
[7]IVI- RMA- Rome, IVI-RMA Rome, Rome, Italy

Study question: Could reproductive outcomes and menopausal symptoms of POI women be improved by autologous stem cell ovarian transplant (ASCOT) or Granulocyte-colony stimulating factor (G-CSF) stem cell-mobilization?
Summary answer: Both ASCOT and G-CSF mobilization promoted follicle growth and raised AFC in 60% POI women; obtaining after COS 4 MII-oocytes, 2 embryos, and 1 pregnancy.
What is known already: Premature ovarian insufficiency (POI) is characterized by oligo-amenorrhea due to follicular depletion in young women, where the only practical option is egg/embryo donation, as their spontaneous pregnancy rate is very low(<4%). We recently described that bone marrow derived stem cell (BMDSC) infusion promotes follicular growth by increasing ovarian vascularization, stromal cell proliferation, and reducing cell death in POI mice models and xenografted human ovarian tissue. Based on this, ASCOT technique has been already tested in poor responders improving ovarian function biomarkers (AMH and AFC) in 81.3% of women and allowing a total of 6 pregnancies and 3 healthy babies
Study design, size, duration: Randomized prospective pilot study started in 2018 at La Fe University Hospital, Valencia. The study involves 20 POI women, according to the following inclusion criteria: ≤38 years, ≥4months oligo/amenorrhea, serum FSH>25IU/l.

Patients were randomized into two study arms: 1) Mobilization of bone marrow stem cells to peripheral blood by a 5-day treatment with G-CSF (10ug/kg/day subcutaneous injection), where cells remain circulating; 2) ASCOT: after G-CSF mobilization, stem cells were collected and transplanted into one ovary.
Participants/materials, setting, methods: To date, 10 patients have been included, 4 of them were randomized to the G-CSF arm and 6 to the ASCOT. After intervention, patients were monitored during 6-month for endocrine function, serum levels of FSH, AMH and estradiol, antral follicle count (AFC) was assessed by ultrasound scan. Patients' basal levels were established before treatment.

When follicular growth was detected (AFC increased in at least 1 follicle) controlled ovarian stimulation was initiated following standard protocols.
Main results and the role of chance: Preliminary results in 10 patients, showed follicular development in both arms when compared to basal levels; these follicular growth waves were detected 90-140 days after treatments.

In the G-CSF group, AFC increased in 50% of recruited patients (2/4) while in the ASCOT this improvement was detected in 66.6% of women (4/6).

Statistically significant decrease of FSH levels was not recorded, but two women in the G-CSF group(50%) and 1 in the ASCOT(16%) showed a two-fold decrease, concluding the follow-up period(82.7±8.1 to 24.1±17.4 and 34.9 to 18.1; respectively).

After G-CSF mobilization, 2/4 women, initiated COS, with a total of 4 punctured follicles, 2 MII obtained and 1 embryo vitrified. Embryo transfer was performed but pregnancy was not achieved; this patient currently has regular menses after the follow-up period. In the ASCOT group, 4 of 6 patients initiated COS cycles. Oocyte pick-up was performed in 70% of initiated COS; where 3 MII and 1 GV were retrieved. One 3-day embryo was vitrified and transferred, having an ongoing pregnancy(20 weeks). These women were unable to undergo COS/oocyte pick-up before treatments due to absence of antral follicles.

Within menopausal symptoms, hot flashes and vaginal dryness improved in 50% of women, while 40% recovered menses (spotting).
Limitations, reasons for caution: These are descriptive preliminary results obtained in the first 10 recruited women that should be confirmed at the end of the trial. Our study lacks from a real control group, without treatment, though poor prognosis POI patients should not be left untreated, as their only reproductive option is egg donation.

Wider implications of the findings: ASCOT in POI women can be a potential therapy for women with no option of having offspring with their own oocytes. This study has shown a future use of stem cells in POI women, even without direct ovarian infusion, which suggest potential development of less invasive procedures in the future.
Trial registration number: NCT03535480

SELECTED ORAL COMMUNICATIONS

SESSION 22: UPDATES ON ART OUTCOMES, BARRIERS AND PREDICTIONS: AN INTERNATIONAL OVERVIEW

06 July 2020 Parallel 6 15:15 - 16:35

O-089 Predicting cumulative livebirth from the second complete cycle of IVF: a population-based study of 49,314 couples

M. Ratna[1], S. Bhattacharya[2], D. McLernon[1]

[1]University of Aberdeen, Institute of Applied Health Sciences, Aberdeen, United Kingdom ;
[2]University of Aberdeen, School of Medicine- Medical Sciences and Nutrition, Aberdeen, United Kingdom

Study question: Can we develop an IVF prediction model to estimate the chance of cumulative livebirth in couples embarking on a second complete cycle of IVF?
Summary answer: Yes, our prediction model can estimate individualised chances of cumulative livebirth over three additional complete cycles.
What is known already: Existing models can estimate the individualised cumulative chance of livebirth following one or more complete IVF cycles in couples embarking on their first IVF treatment. There are currently no prediction models in IVF which can estimate the individualised cumulative chance of livebirth over multiple complete cycles in women who decide to continue IVF after completing their first cycle. Existing cumulative prediction models also fail to account for couples who decide to stop treatment after a complete cycle. Models that do not adjust for discontinuation may give predictions that are too optimistic.
Study design, size, duration: For model development, a population-based cohort was used of 49314 women treated in IVF clinics across the UK from 1999 to 2008 using their own oocytes and their partners' sperm. Model external validation was performed on data collected from 36170 women who underwent treatment in UK IVF clinics from 2010 to 2017.
Participants/materials, setting, methods: All UK IVF treatments were obtained from the Human Fertilisation and Embryology Authority (HFEA) database. Using a discrete time logistic regression model, we predicted the cumulative probability of livebirth from the second to the fourth complete cycle. We adjusted for couple characteristics at the second cycle and outcome of first cycle. Inverse probability weighting was used to account for treatment discontinuation. Discrimination was assessed using c-statistic and calibration was assessed using calibration-in-the-large and calibration slope.
Main results and the role of chance: Of 49314 women, 12408 (25.2%) had a livebirth after their second complete cycle. Cumulatively, 17394 (35.3%) had a livebirth over three further complete cycles. Women who had a livebirth in their first complete cycle had over twice the odds of a livebirth compared to women who did not get pregnant in their first complete cycle (Odds Ratio: 2.18 (95% Confidence Interval: 2.15, 2.21)).

As an example, consider a 32-year-old woman with two years of unexplained infertility who had a livebirth after her first complete cycle. If she starts her second complete cycle one year later, she has a 48% predicted chance of a second livebirth resulting from this second complete cycle. Over three complete cycles her cumulative predicted chance of livebirth is 75%. For a woman with similar characteristics who suffered a pregnancy loss in her first complete cycle the cumulative predicted chance of livebirth is 72% while for a woman who did not get pregnant it is 66%.

The c-statistic for the development and validation cohorts were 0.647 (0.641, 0.652) and 0.626 (0.612, 0.632) respectively. Both calibration-in-the-large (Intercept: 0.0351 (0.0149, 0.0553)) and calibration slope (0.804 (0.769, 0.840)) showed under-prediction in validation cohort. However, after recalibration the fit was much improved.
Limitations, reasons for caution: We were unable to adjust for some potentially important predictors, e.g. body mass index (BMI), smoking and alcohol intake in women, as well as measures of ovarian reserve such as antral follicle count. These were not available in the linked HFEA dataset.
Wider implications of the findings: This is the first model to estimate the overall chance of a livebirth over three further cycles for a couple who have already undergone a complete IVF cycle. The model can be used as a counselling tool to help couples make an informed decision regarding further treatment.
Trial registration number: N/A

O-090 Assisted reproductive technology in Africa: a five-year analysis of data from the African Network and Registry

L. Potgieter[1], P. Archary[1], I. Smit[1], O. Ashiru[2], E. Gwet-Bell[3], S. Dyer[1]

[1]Groote Schuur Hospital, Obstetrics and Gynaecology, Cape Town, South Africa ;
[2]Medical ART Centre, Not applicable, Lagos, Nigeria ;
[3]Clinique Médicale Odyssée, Not applicable, Douala, Cameroon

Study question: What were utilization, outcomes and practices of assisted reproductive technology (ART) in Africa between 2013 and 2017?
Summary answer: Utilization remained low; fresh autologous cycles predominated, with favorable pregnancy rates but accompanied by high rate of multiples and low reporting of delivery outcomes.
What is known already: Global access to infertility treatment requires global understanding of regional realities, challenges and differences. Many African countries, the majority of which are low and lower-middle income countries, carry a triple burden of infertility, namely high infertility disease prevalence, negative socio-cultural consequences, and poor access to effective treatment. Tubal and severe male factor are the leading causes of infertility in Africa, often requiring ART. Availability of ART has been low and treatment outcomes and practices were not monitored regionally prior to 2013. The African Network and Registry for ART (ANARA) commenced data monitoring in 2013 with increasing participation.
Study design, size, duration: Retrospective registry data pertaining to the years 2013 - 2017 were collected cross-sectionally over a period of three years. All ART centres known to exist on the African continent were invited to participate through mailing campaigns, and in conjunction with local workshops and conference presentations. ANARA online software or standardized data forms adjusted from the International Committee for Monitoring ART were used for data collection. Both retrospective summary and cycle-based data were collected and analysed.
Participants/materials, setting, methods: Centre participation and data submission were voluntary. Data sets were received and if necessary, transferred to standard data forms. All data were checked for mathematical errors and corrected as far as possible but not further validated. Data were pooled by year, country and then at regional (Africa-wide) level; and analyzed using descriptive statistics. Most results and trends were reported at regional level only, with the exception of number of procedures and ART utilization.
Main results and the role of chance: Registry participation increased from 40 ART centres in 13 countries in 2013 to 47 centres in 17 countries in 2017. Data are based on 153 461 procedures. Fresh autologous IVF/ICSI predominated in over 70% of procedures, with autologous frozen embryo transfer and fresh and frozen oocyte donation accounting for 17% and 7% respectively. ICSI was performed in over 90% of autologous cycles. Fresh embryo transfer was favoured overall.

The transfer of two and three embryos predominated for all years and all procedures. This practice remained constant with no observable increase in single embryo transfers. There was however a trend towards fewer transfers of four and more embryos. The majority of autologous cycles were conducted in women younger than 35.

During the period of observation, the average pregnancy rate per aspiration was 33% for fresh IVF/ICSI, while the pregnancy rate per thaw in frozen autologous cycles fluctuated between 25% and 39%. These rates were accompanied by a mean multiple delivery rate of 27% and 23% respectively. Overall, only 61% of pregnancies were followed up to delivery.

The evaluation of cycle-based data demonstrated a profound difference between elective and non-elective single embryo transfer in terms of pregnancy rates (47% vs 22%).

Limitations, reasons for caution: Registry participation varied, limiting data representation in some countries and for the continent overall. There were large variations regarding completeness of data. Means to validate data have not yet been established.
Wider implications of the findings: ART monitoring has been successfully initiated in Africa. Efforts are now directed at expanding registry participation, increasing capacity for the collection of prospective cycle-based data with use of appropriate software, and improving pregnancy follow up. Registry data are essential for improvement strategies regarding access to and quality of care.
Trial registration number: Not applicable

O-091 Pregnancy outcomes and lifetime fecundity – a nationwide, registry-based cohort study

A.M. Kolte[1], D. Westergaard[2], Ø. Lidegaard[3], S. Brunak[2], H.S. Nielsen[4]

[1]Copenhagen University Hospital Rigshospitalet, Recurrent Pregnancy Loss Unit- Fertility Clinic 4071, Copenhagen, Denmark ;
[2]University of Copenhagen, Novo Nordisk Foundation Center for Protein Research, Copenhagen, Denmark ;
[3]Copenhagen University Hospital Rigshospitalet, Dept. Gynaecology 4231, Copenhagen, Denmark ;
[4]Copenhagen University Hospital Hvidovre, Dept. Gynaecology and Obstetrics, Hvidovre, Denmark

Study question: How do different pregnancy outcomes and maternal age at first pregnancy influence the predicted total number of live born children (lifetime fecundity)?
Summary answer: A first ectopic pregnancy or pregnancy loss significantly reduced lifetime fecundity. Recurrent pregnancy loss (≥3 consecutive losses) also reduced lifetime fecundity.
What is known already: Several studies have investigated the impact of pregnancy loss, ectopic pregnancies, molar pregnancies and still birth on the next pregnancy. To our knowledge, no large-scale studies have described the effect of prior pregnancy outcomes on lifetime fecundity.
Study design, size, duration: Nationwide register-based cohort study comprising 458,475 women with at least one pregnancy in the Danish Medical Birth Registry or the Danish National Patient Registry from 1977 until 2017.
Participants/materials, setting, methods: We investigated lifetime fecundity in all women who lived in Denmark between their 20th and 45th birthday who had been pregnant at least once. Analyses were based on age at first pregnancy, outcome of the first pregnancy (live birth, ectopic pregnancy, pregnancy loss, still birth or molar pregnancy) and number of pregnancy losses, both total number and maximum number of consecutive pregnancy losses. Lifetime fecundity was estimated using a Generalized Linear Mixed Model.
Main results and the role of chance: Any adverse outcome in the first pregnancy significantly reduced lifetime fecundity compared to live birth. A first ectopic pregnancy had the largest effect (1.16 children on average, 1.11-1.22, 95% CI) compared to a woman with a first live (1.95 children on average, 1.86-2.03, 95% CI), both with the first pregnancy at age 30. Recurrent pregnancy loss (≥3 consecutive losses) also decreased lifetime fecundity significantly (1.57 children on average, 1.5-1.65, 95% CI) compared to women with no pregnancy losses (1.92 children on average, 1.83-1.97, 95% CI). Conversely, the total number of pregnancy losses among women <35 year at first pregnancy had no impact. Sex of the first-born child did not impact lifetime fecundity.
Limitations, reasons for caution: It is a limitation that pregnancy losses handled outside of hospitals are not registered in the Danish registries. Other factors other than pregnancy history likely also influence lifetime fecundity.
Wider implications of the findings: In this unique dataset, we show that adverse pregnancy outcomes have a significant impact on lifetime fecundity. This highlights the importance of taking prior pregnancy outcomes into account in family planning, and the necessity of more research into the pathophysiology of ectopic pregnancies and recurrent pregnancy loss.
Trial registration number: N/A

O-092 Is the number of oocytes retrieved associated with time to conception leading to live birth? A population-based analysis of 221,073 cycles

Y.J. Law[1], N. Zhang[1], R. Paul[1], O. Fitzgerald[1], K. Harris[1], G. Chambers[1], C. Venetis[1]

[1]University of New South Wales, Centre for Big Data Research in Health, Sydney, Australia

Study question: Does retrieving a higher number of oocytes after ovarian stimulation prolong the time to conception leading to live birth in women of different ages?
Summary answer: Retrieval of higher numbers of oocytes does not prolong, but rather, shortens the time to conception leading to live birth in women of all ages.
What is known already: Higher numbers of oocytes retrieved have been shown to be independently associated with more euploid embryos, subsequently leading to higher cumulative live birth rates. However, the euploidy rate (euploid/total embryos available) may vary depending on the number of oocytes retrieved and female age. With more oocytes and eventually embryos available for transfer, particularly in older women, there may be a lower chance of selecting a euploid embryo and therefore, an increased time to conception leading to live birth (TCLB). Whether the number of oocytes retrieved is associated with TCLB has never been investigated.
Study design, size, duration: This is a large retrospective population-based cohort study using data from the Australian and New Zealand Assisted Reproduction Database. Overall, 116,579 women undergoing 221,037 autologous aspiration cycles between January 2009 to December 2015 were included in the analysis. All fresh and frozen embryo transfers resulting from the associated aspiration cycle were included in the analysis until one live birth occurred or all embryos were used. Cycles with no oocytes retrieved were excluded from this analysis.
Participants/materials, setting, methods: Time to conception leading to live birth was assessed in two ways: the number of days (from oocyte pick-up to the date of confirmation of pregnancy) and the number of intended embryo transfers needed to achieve a live birth. Competing risks regression analysis and cumulative incidence functions were used to evaluate the association between oocyte yield and the TLCB. All analyses were also stratified by female age while controlling for important confounders.
Main results and the role of chance: Utilising time in days as the time variable, the number of oocytes retrieved remained a significant positive predictor of TCLB after adjusting for female age, parity, type of embryo transferred, cycle count, insemination method and type of infertility. Compared to the reference group of 10-14 oocytes retrieved, the subdistribution hazard ratio (SHR) of achieving a conception leading to a live birth increased with the number of oocytes retrieved across all age groups. Indicatively, for women aged 35-39, the SHRs (95% CIs) were 1-3 oocytes: 0.44 (0.42-0.47), 4-9 oocytes: 0.71 (0.69-0.73), 10-14 oocytes: 1.00, 15-19 oocytes: 1.16 (1.12-1.20), 20-24 oocytes: 1.35 (1.29-1.41), ≥25 oocytes: 1.41 (1.33-1.50). When the number of intended embryo transfers was used as the time variable, SHRs (95% CIs) were 1-3 oocytes: 0.46 (0.43-0.48), 4-9 oocytes: 0.72 (0.71-0.74), 10-14 oocytes: 1.00, 15-19 oocytes: 1.14 (1.11-1.18), 20-24 oocytes: 1.32 (1.26-1.37), ≥25 oocytes: 1.37 (1.30-1.44). The cumulative incidence curves for all female age subgroups supported that with higher number of oocytes there is a higher cumulative incidence of conception leading to live birth at all time points (either using days or intended embryo transfers as the time variable). A sensitivity analysis including only term live births also yielded similar results.
Limitations, reasons for caution: This study is based on retrospective observational data, thus not all confounders or clinical decisions may be accounted for. Furthermore, causality should not be inferred using these data.
Wider implications of the findings: This study demonstrates that retrieval of higher numbers of oocytes not only does not prolong the TCLB, but instead, seems to lead to higher cumulative incidence of conception leading to live birth in all female age groups. This information is important in informing future clinical decisions and furthering patient education.
Trial registration number: Not applicable

O-093 Variability and ovarian dysfunction of the menstrual cycle – a prospective analysis of the German Natural Family Planning database including 43,999 menstrual cycles

P. Herrmann[1], T. Freundl-Schuett[2,3], L.M. Wallwiener[3], P. Frank-Herrmann[1,3], T. Strowitzki[1,3], A. Freis[1,3]

[1]Universitäts-Frauenklinik, Department of Gynecologic Endocrinology and Fertility Disorders, Heidelberg, Germany ;
[2]Heinrich Heine University Duesseldorf, Department of Obstetrics and Gynaecology, Duesseldorf, Germany ;
[3]German Society of Gynecological Endocrinology and Fertility Medicine DGGEF e.V., Section Natural Fertility, Heidelberg, Germany

Study question: To evaluate if individual variability of cycle length and ovulation, as well as the presence of luteal phase deficiency are more frequent than currently assumed

Summary answer: Intraindividual cycle length as well as fertility parameters differ distinctly subject to age, emphasizing the importance of individual assessment during the ongoing cycle.

What is known already: As the chances to conceive spontaneously vary dramatically during the menstrual cycle, determination of peak fertility remains crucial for couples trying to conceive. Recent reviews and guidelines have shown that there is still a lack of knowledge concerning the spontaneous menstrual cycle. Moreover, the American Society for Reproductive Medicine scrutinizes the clinical relevance of luteal phase deficiency in the etiology of infertility. The symptothermal method combines observation of the periovulatory temperature rise and cervical mucus changes and determines ovulation, as well as the onset and the end of the fertile phase according to the double-check principle.

Study design, size, duration: 43,999 menstrual cycle charts of 1923 women aiming for conception or contraception were collected prospectively between 1985-2019. Ovulation and the fertile window were determined applying the Sensiplan method, using estrogen- as well as progesterone-parameters and an evidenced symptothermal algorithm based on the extended Holt-rules. Ovulation was determined as the day before temperature rise. Luteal phase deficiency (LPD) was defined as hyperthermic phase < 10 days and anovulation as the presence of a monophasic temperature curve.

Participants/materials, setting, methods: Patterns of menstrual cycles (MC; n=12,612 cycles of 1051 women) were analyzed after excluding the use of oral contraception within the last three months. We included women aged 18 - 44 years who contributed data consisting of 12 cycle charts. Women after breast feeding, birth or miscarriage were included as soon as the first ovulatory cycle with sufficient luteal phase was detected. Statistical analysis was performed using single factor variance analysis, chi-squared-tests and logistic regression.

Main results and the role of chance: Mean cycle length (CL) was 29.7±7.6 days (d). 62.5% of women had cycle length variation (CLV) of >7d within 12 cycles. Respective to age, women aged 35-39 years (y) presented with the lowest amount of CLV>7d (52.2%), whereas women aged 18-24y showed CLV in 67.0%. Ovulation occurred most frequently on d15 (12.8%). 54.8% of women had a difference of >7d between the earliest and the latest day of ovulation, with a significant decrease subject to age (18-24y: 62.2%; 25-29y: 56.5%; 30-34y 58.2%; 35-39y: 40.5%; 40-44y: 30.2%, p<0.001). Average luteal phase length was 12.1±2.3d. 11.2% of MC presented with luteal phase deficiency (LPD). 17.6% of women had ≥3 cycles with LPD, whereas 46.9% did not show any LPD. Presence of LPD decreased according to age: 14.4% (18-24y) vs. 8.3% (35-39y) (OR=0.983, 95%CI: 0.972-0.994, p=0.002). Incidence of LPD rose constantly if ovulation occurred later (OR=1.084, 95%CI: 1.074-1.095, p<0.001). Cycles with an ovulation later than d22 showed LPD in 25.8%. Anovulation occurred in 2.6% of MC, irrespective of age. In cycles consisting of <25d or >35d, relative amount of anovulatory cycles increased up to 8.6% and 6.9%, respectively. Ovulatory cycles with sufficient luteal phase occurred in 61.0% (CL<25d), 88.9% (CL=25-35d) and 82.0% (CL>35d).

Limitations, reasons for caution: Data regarding possible comorbidities, such as hyperprolactinemia, were not collected. However, to reduce the impact of possible confounders, only women who contributed at least 12 cycles were included.

Wider implications of the findings: Individual assessment of the current fertile window is crucial for couples trying to conceive. Our data demonstrate that current innovations (such as the majority of mobile apps) claiming to support conception using a rudimentary calendar method based on previous cycles are not suitable to reliably indicate the fertile days.

Trial registration number: not applicable

O-094 Motivational factors and barriers for infertile patients and their partners to seek consultation and treatment

A. Domar[1], R. Vassena[2], M. Dixon[3], M. Costa[4], E. Vegni[5], J Guiglotto[6], M. Markert[7], J. Boivin[8]

[1]Boston IVF, Domar Center for Mind/Body Health, Waltham, U.S.A. ;
[2]Clinica Eugin, Obstetrics and Gynaecology, Barcelona, Spain ;
[3]University of Toronto, ANOVA Fertility and Reproductive Health, Toronto, Canada ;
[4]International Evangelical Hospital, Reproductive Medicine, Genova, Italy ;
[5]Università Statale di Milano, Dept of Health Sciences, Milan, Italy ;
[6]W2O Group, Marketing Research & Strategic Insights, Philadelphia, U.S.A. ;
[7]Ferring Pharmaceuticals, Health Economics & Outcomes Research, Copenhagen, Denmark ;
[8]Cardiff University, School of Psychology, Cardiff, United Kingdom

Study question: What are the key drivers and barriers for infertile patients and their partners to see an infertility specialist and subsequently undergo treatment?

Summary answer: Patients waited three years seeking infertility diagnosis. There were significant differences in perceived healthcare provider services among respondents seeking treatment versus not seeking treatment.

What is known already: Individuals and couples frequently wait years prior to seeking out medical advice and treatment for infertility, decreasing their chances of treatment success. The causes of delays to diagnosis, treatment and pregnancy are not well established amongst patients or their partners.

Study design, size, duration: An online, international, 30-minute quantitative survey collected data from 15th March–17th May 2019. The study included 1,944 respondents across nine countries: United States, Canada, United Kingdom, France, Germany, Italy, Spain, Australia and China. The survey was developed in English and translated to local language. All translations were validated by national linguists.

Participants/materials, setting, methods: The survey assessed average time to treatment, patient and partner perspectives on the treatment journey, and drivers for, and barriers to, infertility treatment. Participants were either (a) infertile patients (N=1037) or (b) partners to infertile patients (N=907; but not necessarily partners of the patient sample), who were at different stages of the treatment journey. Average age was 35.8 (SD=9.66) years, 56% (N=1095) were female, 67% (N=1119) were married and 91% (N=1773) were heterosexual.

Main results and the role of chance: Across countries, average time to diagnosis was 3.2 years (SD=2.4), followed by an average of 2.0 years (SD=2.1) attempting natural conception before consultation, and an average of 1.6 years (SD=1.4) of treatment before successful couples achieved pregnancy.

The most frequently reported driver for considering treatment in respondents with a consultation (N=1025) was an equal desire within couples to have a child (41%), followed by a willingness to do anything to become parents (38%). Among partners (N=356), 28% cited transparency of information from health care professionals about treatment expectations as an important driver. Of respondents not seeking consultation following diagnosis (N=352), the most frequent reason was perceived cost (38%).

Post consultation, only 32% of respondents not seeking treatment (N=207) reported that their healthcare professional offered services, such as supportive services, compared to 61% of respondents seeking treatment (p<0.001). Similarly, 37% of respondents not seeking treatment agreed that their practitioner had offered mental health services, compared to 62% among those receiving treatment (p<0.001). The most frequently reported barriers for those not seeking treatment were cost (42%, N=87) and determination to conceive naturally (31%, N=64). Of the 95 couples who discontinued treatment, 35% (N=33) discontinued due to financial impact.

Limitations, reasons for caution: This study was an anonymous, quantitative online questionnaire, there was no response validation by healthcare professionals, with potential to be a non-representative sample. Additionally, respondents were unable to ask clarifying questions. Recruitment of individual participants meant it was not possible to run sub-analyses of patient-partner pairs.

Wider implications of the findings: Respondents delayed consultation and treatment for years, which can negatively impact chances of pregnancy. Motivational coherence in the wish for a child was a key driver for treatment. Costs were a main barrier. There were large differences in perceived supportive

service offerings among respondents seeking treatment versus not seeking treatment.

Trial registration number: Not applicable

INVITED SESSION

SESSION 23: RECENT ADVANCES IN ENDOMETRIOSIS

06 July 2020 Parallel 2 17:00 - 18:00

O-095 Management of pelvic pain in women actively trying to conceive

S. As-Sanie[1]

[1]

O-096 Organoids as 3D models for endometrium and endometriosis

H. Vankelecom[1]

[1]*Cluster of Stem Cell and Developmental Biology, Development and Regeneration, Leuven, Belgium*

Abstract text

The endometrium is the first contact site of the embryo and crucial for human reproduction. Mechanisms underlying the tissue's monthly remodeling during the menstrual cycle and its embryo receptivity remain far from understood, as well as how these processes go awry during endometrium pathology. This limited understanding is primarily due to a lack of research models reliably recapitulating endometrium biology and disease in nature and heterogeneity.

Organoid technology provides an innovative tool to grow mini-tissues in culture. We established 3D organoid models from both healthy and diseased endometrium which reproduce key features of the original epithelium. The organoids show long-term expansion capacity while remaining genomically, transcriptomically and functionally stable. The endometrium-derived organoids phenocopy physiological responses to reproductive hormones and mimic the menstrual cycle in a dish. The organoids derived from endometriosis (as well as endometrial cancer) recapitulate characteristics of the patients' diseased tissue and faithfully capture the clinical heterogeneity of the disease. Finally, the endometrial disease organoids reproduce the original lesion when transplanted in immunodeficient mice.

Taken together, we created new organoid models that provide powerful and innovative tools to decipher the mechanisms underlying endometrium biology and pathology, and at the same time serve as screening platform to test (new) drugs, even in a patient-personalized manner.

Trial registration number: -

Study funding: -

Funding source: -

INVITED SESSION

SESSION 24: PROMOTING FERTILITY AWARENESS IN YOUR OWN BACKYARD

06 July 2020 Parallel 3 17:00 - 18:00

O-097 Educate, assess and counsel – who, how and when?

A.N. Andersen[1]

[1]*Copenhagen University Hospital, The Fertility Clinic- section 4071, Copenhagen, Denmark*

Abstract text

The United Nations definition of reproductive health from 1995 stated that people should have "the capability to reproduce and to decide if, when and how often to do so". This was written at a time where focus was on contraception, but the wise phrasings also remains relevant considering today´s challenges in human reproduction, with low total fertility rates (TFR) around 1.5 in Europe. The question today is how we can assist more women and men to fulfil their reproductive life-plans in terms of number and spacing of children.

The lecture will propose a 3-step strategy. **A)** Women and men below 20 years evidently should have their main focus on contraception, but public campaigns and school education should create an initial awareness their fertility and avoidance of sexually transmitted diseases **B)** In the age groups 20 to 30 there should still be a focus on public campaigns, but another effort could be to "integrate pro-fertility thinking" into family planning. To phrase Seifer et al. (2015) by "putting family back into family planning". In family planning individual assessment and counselling is part of the consultation, and it could include simple tools for self-assessment of reproductive risks, like the FertiSTAT colour-coded sheet **C)** In the age of 30 society could offer nulliparous women and men pro-fertility-focussed individualized assessment, education and counselling. Thirty is the age where many nulliparous women really gets motivated for individual pro-fertility guidance.

The lecture will focus on point C, the approach offered in the Fertility Assessment and Counselling Clinic (FAC) that has initially been outlined by Hvidman et al. in 2015. In short, it offers a free 30 minutes consultations with specialist in Reproductive Medicine for singles and couples. Women have assessment of risk factors for impaired fertility graded in green, yellow and red scores, including measurements of antral follicle counts and pelvic pathology by ultrasound, and a blood sample for AMH. The consultations always include review of educational graphs on age and female fertility. Men are reviewed for known risk factors for male subfertility, and a sperm analysis is made "on-site". Couples with known infertility are NOT seen at the FAC clinic, which use the facilities of the Fertility Clinic, but operates separately. Individuals do not need any referral and the activity is funded by the public regional health system. During the last 8 years the clinic has had a total of around 4.000 consultations.

The mean female age is around 33 years. Lower in couples and higher in single women. The main motive for seeking the consultation was that 70% of the females wanted an estimate on how long they could postpone childbearing. Around 40% are single women and 30% use contraception. Follow-up studies have shown that both women and men remembered the consultation several years and that the score sheets to some extend can predict natural conceptions. Many women saw the consultation as a catalyst for change, in general towards advancing their attempts to conceive. The men felt empowered after the fertility counseling because they were equipped with concrete information that could inform their parenthood plans and decisions.

We argue that 30 years is the time where nulliparous women a) consider themselves susceptible to the condition of not being able to achieve their desired family size and b) still have 5 years ahead without major decline in age-related subfertility and c) believes that specific action to become pregnant can help them.

O-098 Getting fertility education on the national curriculum

A. Balen[1]

[1]*The Leeds Centre for Reproductive Medicine, Professor of Reproductive Medicine and Surgery, Leeds, United Kingdom*

Abstract text

Getting fertility education on the national curriculum

Professor Adam Balen

Professor of Reproductive Medicine, Leeds Teaching Hospitals, UK

In 2016, when I was chair of the British Fertility Society I founded the Fertility Education Initiative(FEI) with aim of improving people's knowledge of fertility and reproductive health in the UK. We brought a number of partner organisations to the project, including the RCOG, Faculty of Sexual and Reproductive Healthcare, Sex Education Forum, Sexpression, Teenage Pregnancy Knowledge Exchange, Fertility Network UK, Fertility Fest and Public Health England. Key members of the national committee are the deputy chairs Professor Joyce Harper (Institute for Women's Health, University College London) and Professor Jacky Boivin (School of Psychology, Cardiff University).

We first held a "Fertility Health Summit" which created a lot of publicity in the national media. Our vision is to ensure that people have a greater understanding and awareness about fertility and reproductive health, so they can make an informed choice about their own fertility journey, or that of others they may have an impact on.

In brief, our three key aims are:

1. Understanding human fertility:
   a) Human Reproduction
   b) Male and female reproductive health, including the reproductive life cycle; fertility and infertility; signs, symptoms and preventable causes of fertility issue; and planning for a healthy pregnancy
2. Understanding modern families:
   a) Societal and cultural variations in family building
   b) Routes to parenthood; for heterosexual, LGBTQ+ and single people with and without fertility issues; assisted conception techniques for family building; other routes to parenthood (such as adoption, fostering, step-families); and living a life without children
3. Understanding current reproductive technologies: To help educate people about what reproductive technologies can and cannot do and how they might impact on how human beings are made in the future.

We are developing age-appropriate educational material, some of which is already available on the BFS website and a series of animations, The first Your Fertility Matters https://youtu.be/ETwDCKBaYd4 and the second Fertility Technologies Shaping Modern Families https://youtu.be/dOi08g3CLOc covering modern ways of forming families and routes to parenthood for heterosexual, LGBTQ+ and single people with and without fertility issues.

In 2019, the draft statutory guidance on sex education (now known as Relationship and Sex Education, RSE) and health education was presented to the UK government for debate. This followed a highly publicised public consultation that we contributed to on behalf of the Fertility Education Initiative. We were delighted that the new guidance includes the need to educate young people about 'the facts about reproductive health, including fertility and the potential impact of lifestyle on fertility for men and women'. The bulk of the guidance naturally deals with general health and wellbeing, the foundation of healthy relationships and all aspects of physical, emotional, mental, sexual and reproductive health and wellbeing. There is also reference to understanding the various forms of sexuality and sexual relationships and 'that others' families, either in school or in the wider world, sometimes look different from their family, but that they should respect those differences and know that other children's families are also characterised by love and care'. There is emphasis on age-appropriate information and when specific topics should be discussed.

The inclusion of information on fertility in the guide is a huge step forward for fertility education, which until now has been largely overlooked, poorly taught and not even properly covered in most biology syllabi, let alone PHSE (personal, social, health and economic education) or RSE lessons.

INVITED SESSION

SESSION 25: THE FUTURE OF ANDROLOGY

06 July 2020 Parallel 4 17:00 - 18:00

O-099 Advanced testing, the advantage of additional knowledge

E. Baldi[1], M. Muratori[2], L. Tamburrino[3], S. Marchiani[4]

[1]*University of Florence- Italy, Experimental and Clinical Medicine, Florence, Italy ;*
[2]*University of Florence, Experimental and Clincal Biomedical Sciences, Florence, Italy ;*
[3]*University of Florence, Experimental and Clinical Medicine, Florence, Italy ;*
[4]*University of Florence, Experimental and Clinical Medicine, Florence, Italy*

Abstract text

In order to fertilize and support embryo development, spermatozoa require several functional attributes, most of which cannot be revealed by a routine semen analysis. Indeed, semen analysis reveals only two functional sperm properties needed for fertilization, progressive motility and normal morphology. In vivo, spermatozoa must penetrate cervical mucus, undergo the complex process of capacitation, develop a special type of motility known as hyperactivation necessary to penetrate oocyte vestments, undergo acrosome reaction, fuse with the oolemma and, finally, deliver an intact DNA to the oocyte in order to achieve syngamy. Each of these functions requires the endowment of molecules, receptors, pathways activation, second messengers as well as morphological characteristics of the spermatozoa. Many of these features have been disclosed in the last years, but more research efforts are needed in order to fully understand the complexity of the fertilization process. Based on this knowledge, in the last three decades, tests have been developed to evaluate the sperm ability to accomplish many of the functions required for a correct fertilization, including the integrity of DNA. Some of these tests have potential clinical value both for natural and assisted reproduction, as they predict sperm fertilizing ability and ability to support development of embryos characterized by better quality and implantation with good sensitivity and specificity. However, some of these texts are complex, poorly standardized, expensive and require equipment that may not be present in laboratories for routine semen analysis. Hopefully, progresses in technology will lead to development of easy to perform and inexpensive tests.

O-100 Routine and new diagnostic approaches for male infertility: Are new genetic markers near primetime?

D.J. Lamb[1]

[1]*Weill Cornell Medicine, Urology, New York, USA*

SELECTED ORAL COMMUNICATIONS

SESSION 26: THE DAY AFTER. FERTILITY PRESERVATION AND EMBRYO TRANSFER IN PATIENTS WITH CANCER DIAGNOSIS.

06 July 2020 Parallel 5 17:00 - 18:00

O-101 Fertility preservation in men affected by cancer: a retrospective analysis of their experiences around fertility counseling and evaluation of needs regarding additional support tools.

V. Ehrbar[1], G. Sartorius[1], C. Urech[1], C. Rochlitz[2], S. Tschudin[1]

[1]*University Hospital Basel, Gyn. Socialmedicine and Psychosomatics, Basel, Switzerland ;*
[2]*University Hospital Basel, Medical Oncology, Basel, Switzerland*

Study question: What are the needs of male cancer patients regarding an online information tool based on their experience with fertility preservation?
Summary answer: Male cancer patients consider counselling by professionals regarding fertility preservation as very helpful and would favor complementing support tools.
What is known already: Impairment of fertility is a common sequela of the nowadays available and often highly efficient options to treat cancer. Thus, discussing fertility preservation (FP) with all young cancer patients before start of treatment is crucial. The few existing studies in male cancer patients show that appropriate information about FP increases counseling satisfaction and prevents regrets about decisions regarding FP. According to research in female cancer patients, online decision aids are a helpful and efficient complement to fertility counseling. Support tools for men, however, are scarce and predominantly in English. Little is known about their needs regarding this kind of support.
Study design, size, duration: The study consisted of a quantitative and a qualitative part, i.e a retrospective questionnaire-based cross-sectional survey followed by focus groups. Recruitment was ongoing for 30 months in three fertility centers in Switzerland. Participants had a history of cancer within the last five years and were invited regardless of their decision regarding FP.
Participants/materials, setting, methods: Participants (N=72) completed the online questionnaire specifically developed for the given context. The survey consisted of questions about the experiences and needs of male cancer patients regarding counseling and regarding information provision concerning FP. Subsequently three focus groups (N=12) were conducted, where the topics covered by the survey were discussed more profoundly.
Main results and the role of chance: Mean age of the participants of the online survey was 32.94 (SD 8.38), most of them are living in a relationship

(70.8%) and 30.6% have at least one child. Predominant type of cancer was testicular cancer (55.6%), followed by lymphomas (16.7%) and leukemia (13.9%). All but two participants decided to undergo FP by semen cryopreservation. The moment, when they were informed about FP was for 68.1% of the participants before start of cancer treatment and for 29.2% during treatment, respectively; whereas 2.8% did not recall having been informed at any time. The professional who provided the information was predominantly an oncologist (41.9%) or urologist (30.6%). Participants rated the significance of the counseling as very high with a mean of 4.2 (maximum of 5) and experienced the professionals as supportive (4.37 out of 5). Analysis of the focus groups showed that support tools such as the existing decision aids for women would be welcome. The majority of the participants (70.8%) stated that they would use such a tool, which should ideally comprise structured information provision, patient stories, and deeper insights in topics such as sexuality, fertility as well as contact information to peers and professionals.

Limitations, reasons for caution: Participants answered the questionnaire retrospectively; therefore, a recall bias needs to be considered. The final sample of participants consisted uniquely of patients who got a referral to fertility counselling. Consequently, these results are not generalizable for all male cancer patients.

Wider implications of the findings: These data complement the preexisting studies focusing on female cancer patient's needs regarding FP and support by a male perspective. They highlight that male patients would profit from a support tool, as well. In general, more research is required in the specific domain of psychological impact on male patients.

Trial registration number: NCT03876366

O-102 Single men's attitudes towards posthumous reproduction does not necessarily match accepted social trends.

A. Stein[1], E. Altman[1], M. Rotlevi[1], A. Deutsch[1], Y. Shufaro[2]

[1]Beilinson Medical Center, Sperm bamk and Male infertility, Petah Tikva, Israel ; [2]Beilinson Medical Center, Infertility and IVF Unit, Petah Tikva, Israel

Study question: What's the attitude of single men cryopreserving sperm in the face of serious morbidity/mortality risks to the use of their sperm for posthumous reproduction?

Summary answer: Most of the single men who cryopreserved their sperm in face of a mortality/morbidity risk object to posthumous reproduction using their sperm.

What is known already: Requests for posthumous reproduction are growing, raising an ethical debate and prolonged litigation, especially when written instructions were not left by the patients. The issue of the progenitors' intention to procreate (or not) after death is the key to ethically based decision making. In the Israeli society procreation is a very strong value, so in the absence of written instructions the patients' relatives often claim for posthumous reproduction based on the presumed will of the deceased in accordance to the mainstream social trend.

Study design, size, duration: Prior to sperm cryopreservation, single men were asked to sign a structured form declaring their consent or refusal for usage of their cryopreserved sperm in case of future mortality. Addressing this issue and signing the form after counselling is a mandatory requirement for sperm cryopreservation. Adolescent patients signed the form with their parent / guardian. The patients were from different religious and cultural origins.

Participants/materials, setting, methods: Five-hundred-thirty-four single man; 401 adults and 133 adolescents, referred for sperm cryopreservation prior to medical/surgical treatment in a tertiary hospital. All the adolescents and 296/401 (74%) adults were diagnosed with malignant disorders. The remaining 105 adults were facing fertility endangering procedures. The choices in the form were to destroy the sperm or to allow posthumous use by a female partner or other people of their choice.

Main results and the role of chance: Five hundred thirty four single men signed the form providing instructions in case of mortality. One hundred thirty three were 13-18 years old (age 15.9 ± 1.16 years) and the rest (401) were adults (age 27.4 ± 8.06 years).

Out of the adult group 5 patients (1.2%) authorized their sibling to use their sperm, 23 (5.7%) willed their sperm to their informal female partners, and 16 (4.0%) permitted their parents to use their sperm posthumously. The significant rest 357 (89.0 %) ordered to destroy their cryopreserved sperm in case of their expiry.

Out of 133 adolescence, four (3%) gave consent to their parents to use their sperm in case of death, while 97% (129 cases) ordered to discard their sperm in such a case.

Limitations, reasons for caution: Despite the size of the group and the social diversity of its members, the findings might still reflect a local reality.

Wider implications of the findings: These results indicate that despite the value of parenthood in the Israeli society, most single men cryopreserving sperm in face of morbidity/mortality danger do so for their own future live parenthood are not interested in posthumous reproduction

Trial registration number: Not applicable.

O-103 Considerations regarding (non-)use of frozen oocytes or embryos for embryo transfer after fertility preservation in young breast cancer survivors.

E. Butalid[1], I. Vriens[2], R. Van Golde[3], J. Derhaag[4], B. Van Bree[3], C. De Die - Smulders[5], V. Tjan- Heijnen[2], L. Van Osch[5]

[1]MUMC+, Obstetrics and Gynaecology, Utrecht, The Netherlands ; [2]MUMC+, Oncology, Maastricht, The Netherlands ; [3]MUMC+, Obstetrics and Gynaecology, Maastricht, The Netherlands ; [4]MUMC+, Embryology, Maastricht, The Netherlands ; [5]MUMC+, Genetics, Maastricht, The Netherlands

Study question: What are female breast cancer survivors' considerations regarding (non)use of frozen oocytes or embryos for embryo transfer after fertility preservation prior to breast cancer treatment?

Summary answer: Most women report a strong intrinsic motivation to pursue natural conception. Time pressure to become pregnant was the most mentioned consideration to perform embryo transfer.

What is known already: Fertility preservation is used in young women undergoing chemotherapy for early-stage breast cancer. Several groups have reported on the return rate of women for their cryopreserved oocytes or embryos after fertility preservation, varying from 0% to 33%. Insight into the women's underlying reproductive considerations and their preferred mode of conception in this specific situation, are, however largely lacking.

Study design, size, duration: This qualitative study investigated women's deliberation of reproductive options after fertility preservation and breast cancer treatment. Interviews were planned until saturation of themes had been achieved. Sixteen women and three male partners took part in semi-structured interviews between August 2017 and August 2018.

Participants/materials, setting, methods: Interviews were conducted with women who had oocytes or embryos cryopreserved prior to breast cancer treatment, at the Maastricht University Medical Center between October 2008 and March 2015. To achieve heterogeneity, women who had had embryo transfer, who had tried to conceive without embryo transfer and who had not tried to become pregnant after breast cancer diagnosis were included. Grounded theory approach was used for analysis.

Main results and the role of chance: Mean age at breast cancer diagnosis was 29.5 years (range 23-38). Mean time after diagnosis was 4.6 years (range 2-8). There was a strong intrinsic motivation to pursue natural conception, which was strengthened by psychological, practical and physical considerations. Reported psychological considerations included that trying spontaneously felt more relaxed and would save 'the back-up option' (i.e. the frozen oocytes or embryos). A practical consideration was that hospital visits or medicines were not needed. Time pressure to become pregnant quickly was a major issue among women who considered embryo transfer, which was considered as a faster method. Time pressure was experienced due to interruption of adjuvant endocrine therapy or the feeling that conception was already delayed because of breast cancer treatment. The wish to use pre-implantation diagnosis (PGD) treatment for hereditary breast cancer disease was another consideration to opt for embryo transfer. Furthermore, women considered the physician's advice as a strong influence to choose for either mode of conception. Women did not have any regret regarding the fertility preservation procedure and regarded the presence of oocytes or embryos as a back-up plan as psychologically reassuring.

Limitations, reasons for caution: During the study participants were in different phases of family planning. This possibly is causing recall bias in the reporting of original considerations. Some already completed their family for some years. Others were still using adjuvant endocrine therapy and may not oversee future considerations.

Wider implications of the findings: The lack of regret and the psychological reassurance of a back-up plan could be supportive for women who opt for fertility preservation. Since time pressure and PGD were the most reported considerations for embryo transfer, well-thought-out counseling about the mode of conception should be done by a reproductive specialist.
Trial registration number: n/a

O-104 Development and testing of an online fertility preservation decision aid for female cancer patients

M. Van den Berg[1], C. Beerendonk[1], A. Bos[2], M. Boshuizen[3], D. Determann[3], R. Van Eekeren[4], C. Lok[5], E. Schaake[6], E. Witteveen[7], M. Wondergem[8], D. Braat[1], R. Hermens[9]

[1]Radboud University Medical Centre, Obstetrics and Gynaecology, Nijmegen, The Netherlands ; [2]University medical center Utrecht, Obstetrics and Gynaecology, Utrecht, The Netherlands ;
[3]Patientplus, Medical writer, Utrecht, The Netherlands ;
[4]Rijnstate hospital, Surgical oncology, Arnhem, The Netherlands ;
[5]Antoni van Leeuwenhoek, Gynaecology, Amsterdam, The Netherlands ;
[6]Antoni van Leeuwenhoek, Radiotherapy, Amsterdam, The Netherlands ;
[7]University medical center Utrecht, Medical oncology, Utrecht, The Netherlands ;
[8]Amsterdam UMC- locatie VU, Hematology, Amsterdam, The Netherlands ;
[9]Radboud University Medical Centre, IQ Healthcare, Nijmegen, The Netherlands

Study question: What are female cancer patients' experiences with an online developed fertility preservation decision aid (DA) tailored to cancer type and associated cancer treatments?
Summary answer: Female cancer patients considered the DA very helpful in decision-making, particularly the personalized information and the value clarification sheet for recognizing personal values in decision-making.
What is known already: Decision-making regarding future fertility is very difficult and complex for female cancer patients. The decision has to be made in a very short time frame in a period with great emotional distress. As a consequence, patients experience decisional conflict regarding this decision. To support female cancer patients and to decrease their decisional conflict, fertility preservation (FP) DAs are being developed. However, in order to make a well-informed decision, patients need personalized information tailored to their cancer type and treatment. DAs that provide information based on cancer type and treatment are not available yet.
Study design, size, duration: The DA was systematically developed by a multidisciplinary steering group in 2019 using recommendations published by Coulter and the International Patient Decision Aid Standards. Patients' and professionals' needs in decision-making were identified by in-depth interviews with female cancer survivors and oncofertility professionals. Patients' experiences with the DA were evaluated with semi-structured interviews with female cancer survivors by using the think aloud method and by questioning the acceptability, usability, comprehensibility, and readability of the DA.
Participants/materials, setting, methods: The DA steering group (N=21) consisted of representatives of healthcare professionals working in female oncofertility care throughout the Netherlands, patient association representatives (adolescent and young adult, breast, gynaecological and haematological cancer), researchers with expertise in shared decision-making and medical writers. After an iterative process of reviewing and revising with the steering group, the DA was evaluated with female cancer survivors recruited from a tertiary hospital and patients' associations.
Main results and the role of chance: Patients and professionals expressed a need for information tailored to cancer type with associated cancer treatments and infertility risks in the DA. Furthermore, the following information was considered important: infertility risks associated with cancer treatment; burden/risks and pregnancy chances of fertility preservation treatment; consequences of the decision for future fertility; and patients' personal values in decision-making. These preferences complemented with the national fertility preservation guideline formed the basis for the development of the DA.

Several face-to-face and online meetings were held with the steering group to discuss and review the structure and content. After reaching consensus, 17 female cancer survivors and patients' representatives were interviewed while using the DA.

Patients were satisfied with the content and lay-out of the DA and considered it very helpful in decision-making. In particular, the cancer-specific information and the value clarification sheet for recognizing their personal values was of great value. All patients would have liked to use the DA if this was available. To improve the DA, the following was suggested: clarify navigation through DA, make tables and graphs more readable, personalize DA more by letting choose which FP options to read about, and emphasize that the DA does not replace oncofertility consultations.
Limitations, reasons for caution: Bias could have occurred because most interviewed patients had a strong wish to conceive prior to decision-making. Patients who have doubts about their wish to conceive and refrain from a treatment may make their decisions based on different information and values.
Wider implications of the findings: After revising the DA according to the improvement suggestions, the tool is ready for implementation into routine clinical practice. Future studies should evaluate if the DA reduces decisional conflict and decision regret regarding fertility preservation decision-making.
Trial registration number: not applicable

INVITED SESSION
SESSION 27: FRONTIERS IN DEVELOPMENTAL BIOLOGY

06 July 2020 Parallel 6 17:00 - 18:00

O-105 The mRNA translational program and the control of nuclear and cytoplasmic events

M. Conti M.D.[1], F. Franciosi[2], N. Costermans[3]

[1]UCSF, Department of OB/GYN- CRS, San Francisco- CA, U.S.A.
[2]Reproductive and Developmental Biology Laboratory, Department of Health- Animal Science and Food Safety- University of Milan, Milan, Italy
[3]Center for Reproductive Sciences, Department of Obstetrics Gynecology and Reproductive Sciences, San Francisco, U.S.A.

Abstract text

The mRNA translational program and the control of nuclear and cytoplasmic events 20/02/2020: Changed by ESHRE Chairs

After a prolonged phase of growth and accumulation of cytoplasmic and nuclear components, a fully-grown mammalian oocyte undergoes a series of more rapid changes in preparation for fertilization and acquisition of totipotency. All these changes are thought to be essential to produce a "good quality egg" that supports embryo development after fertilization. Although the events associated with nuclear maturation are relatively well described and linked to generation of an egg of the correct ploidy, much less is known about the molecular changes in the cytoplasm of an oocyte. To investigate these processes, we have focused on the program of maternal mRNA translation taking place during meiotic maturation and at the oocyte-to-zygote transition. Since transcription is silent at these stages, gene expression is entirely dependent on RNA translation. Taking advantage of a novel RNAseq strategy to quantify mRNA translation, we have generated a blueprint with genome-wide resolution of the changes in translation during both mouse oocyte growth and meiotic maturation. Analysis of these data show that a switch in the pattern of maternal mRNA translation takes place at the time of oocyte reentry into the meiotic cell cycle. Translation of mRNAs coding for proteins required during oocyte growth, including those involved in ribosome and mitochondrial biogenesis, ceases. Conversely, translation of mRNAs for cell cycle components, for chromatin remodeling, and for the transcription machinery that will be used in the embryo becomes activated. Preliminary data show that similar regulation occurs in human oocytes although with different timing. Translational inhibition of specific mRNAs including those coding for the histone variant H3.3 causes delayed developmental defects in the zygote and in the early embryo. In the same vein, defective translation of cell critical cycle components yields aneuploid oocytes and compromised pregnancy. We show that oocyte manipulations known to decrease oocyte quality, including denudation or disruption of endocrine or paracrine signaling in the somatic compartment, also affect the program of maternal mRNA

translation. Remarkably, preliminary data strongly suggest that translation of maternal mRNAs is also disrupted in oocytes during maternal aging. Taken together, these data demonstrate that execution of the maternal mRNA translation program during oocyte maturation is essential to produce a good quality egg and suggest that translational defects are a cause of the compromised developmental competence observed during maternal aging. Supported by NIH P50 HD055764 and R01 GM116926.

O-106 Extended in vitro culture of human embryos beyond the implantation stages

M.Popovic[1]

[1]*Eugin Clinic, Barcelona, department of research and development, Spain*

human reproduction

ESHRE 2020 / Oral presentations

INVITED SESSION

SESSION 28: REVISITING EARLY EMBRYO DEVELOPMENT

07 July 2020 Parallel 1 08:30 - 09:30

O-107 Dynamics of the epigenetic landscape in blastocyst development and stem cell models

P. Rugg-Gunn[1]

[1]*Babraham Institute, Epigenetics, Cambridge, United Kingdom*

Abstract text

Epigenetic processes are essential for regulating the spatiotemporal patterns of gene expression in development. Upon fertilisation, terminally differentiated gametes undergo extensive and genome-wide epigenetic reprogramming as they become totipotent zygotes. These events establish distinctive chromatin states and are thought to enable the efficient activation of zygotic transcription. As the embryo develops, differences in chromatin patterns are established between embryonic and extraembryonic tissues that reinforce lineage specification decisions. Excitingly, the recent development of genome-wide, epigenetic profiling methods that can examine low cell numbers, including single cells, have opened up the possibility of directly investigating the links between chromatin states and lineage specification and also the molecular basis for proposed mechanisms such as lineage priming.

Another exciting development in the field, and one that complements our expanding knowledge of human embryos, is the progress that has been made in using human pluripotent stem cells (hPSCs) as in vitro models to understand the early stages of human embryo development. Recent work has captured hPSCs in multiple states that are broadly termed naïve and primed. Both cell states can self-renew, but are functionally and molecularly distinct. Naïve hPSCs largely recapitulate the transcriptome and epigenome of pre-implantation embryos, and primed hPSCs are similar to early post-implantation embryos. This is an important distinction because these two developmental stages differ enormously in gene regulation and in epigenetic hallmarks such as X-inactivation status and DNA methylation. The research in my laboratory is focused on understanding the mechanisms of epigenetic and gene regulatory changes as hPSCs transition between the two states, with the aim of applying that information to more precisely control cell fate decisions and to better understand human development. Some of our work has also uncovered major differences in developmental strategies that exist between humans and other species in the control of epigenetic events, such as X-chromosome regulation. I will discuss our recent progress on these topics in my seminar.

O-108 Cell cycle in the preimplantation embryo: mechanisms, errors, consequences and cell fate

J. Vermeesch[1]

[1]*Leuven University hospital, department of Human Genetics, Leuven, Belgium*

INVITED SESSION

SESSION 29: BUILDING BRIDGES TOWARDS HARMONISATION

07 July 2020 Parallel 2 08:30 - 09:30

O-109 ESHRE as partner in current and future EU projects

K. Lundin[1]

[1]*Sahlgrenska University hospital, Reproductive Medicine, Göteborg, Sweden*

O-110 The future of EU legislation on tissues and cells

D. Fehily[1]

[1]*European Commission, DG SANTE, Brussels, Belgium*

INVITED SESSION

SESSION 30: NURSE OR MIDWIFE LED E-HEALTH CARE INTERVENTIONS

07 July 2020 Parallel 3 08:30 - 09:30

O-111 Development and evaluation of a preconception health online learning module for nurses in primary care

K. Hammarberg[1]

[1]*Monash University, School of Public Health and Preventive Medicine, Melbourne, Australia*

Abstract text

It has long been known that a woman's health and health behaviours during pregnancy are crucial for pregnancy health and the health of the newborn. There is now growing evidence that poor maternal and paternal health before conception can result in difficulties conceiving, pregnancy complications, impaired fetal growth, poor birth outcomes, and short and longer-term health problems for children [1].

Nurses and midwives who provide primary health care are ideally placed to opportunistically discuss pregnancy intention and promote preconception health with people of reproductive age. However, we have established in two separate studies conducted in collaboration with nurses and midwives, that although nurses and midwives believe it is part of their role to promote fertility and preconception health in their practice, they rarely do so because they lack the knowledge, skills, and resources to initiate conversations about optimising health before conception [2, 3]. Almost all nurses and midwives in these studies agreed that more information and education about preconception health would increase their confidence to discuss it with their patients.

'Your Fertility' is an Australian government funded fertility and health promotion program. In collaboration with the Australian Primary Health Care Nurses Association (APNA), and using co-design where nurses and midwives contributed to the development work, members of 'Your Fertility' developed an evidence-based online learning module to assist nurses and midwives working in primary health care settings promote preconception health in their practice. We are currently conducting an evaluation where participants complete a brief online survey before and after completing the learning module. This will allow us to assess if the online learning module improves knowledge about and attitudes towards promoting preconception health in clinical practice. The second survey will also include questions about participants' perceptions of the acceptability, salience, and comprehensibility of the learning module.

In my presentation I will describe the development and evaluation of this preconception health online learning module for nurses in primary care.

1. Stephenson J, Heslehurst N, Hall J, Schoenaker DAJM, Hutchinson J, Cade JE, Poston L, Barrett G, Crozier SR, Barker M et al. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. The Lancet 2018;391: 1830-1841
2. Hammarberg, K., et al., Knowledge, attitudes and practices relating to fertility among nurses working in primary health care. Australian Journal of Advanced Nursing, 2016. 34(1): p. 6-13.
3. Hammarberg, K. and L. Taylor, Survey of Maternal, Child and Family Health Nurses' attitudes and practice relating to preconception health promotion. Australian Journal of Primary Health, 2019. 25(1): p. 43-48.

O-112 The midwife-led development of e-based pre-conception care programme

I. Delbaere[1]

[1]*VIVES University of Applied Sciences, Health care, Kortrijk, Belgium*

Abstract text

As in most European countries, a large proportion of Flemish women start to late with the intake of folic acid preconceptionally. A study of Hoppenbrouwers et al. addressing this issue, was the motivation for the Flemish Minister of Welfare, Public Health and Family to launch a website on preconception care 'gezondzwangerworden.be' (getting pregnant healthy) in 2015.

For the preparation of this website, preconceptional and prenatal guidelines were assessed by means of AGREE II. Topics for the website were selected by an internal committee of 5 experts and an external committee of 16 experts. Content was developed, based on guidelines and content validation was carried out by 40 experts.

When the website was launched, there was press communication and professional societies of midwives, obstetricians and family medicine were informed. Unfortunately, there were no resources for large communication strategies. Nevertheless, the website is visited by a growing number of couples and health care providers (currently 800 visitors daily). However, the intended effect on folic acid intake remains unclear.

Recently, the website was transformed formally. An included lifestyle test enhances interaction with visitors and enables tailored advise. Although this website with preconception advise includes information about the effect of age on fertility, this is not the most optimal platform to increase fertility awareness, in that couples visiting the website mostly already decided to start a family. In order to inform people about the impact of age on fertility, we developed an interactive tool 'klaarvoorkinderen.be' (ready for children). The aim of this tool is to encourage individuals and couples to reflect about parenthood on the one hand and to inform them on the other hand. The tool was developed in cooperation with professionals of different disciplines and was finetuned after focus group discussions with the target audience. Within these focus groups in different age groups, men and women were involved, as well as people with a desire to start a family and people who did not want children.

In Europe, a growing number of e-based preconception tools with evidence-based information is now available to inform the public about optimal preparation for a healthy pregnancy. These websites can be useful for healthcare providers as well, as a tool of guidance in preconception consultations.

SELECTED ORAL COMMUNICATIONS

SESSION 31: PREDICTIVE ALGORITHMS IN CLINICAL EMBRYOLOGY

07 July 2020 Parallel 1 10:00 - 11:30

O-113 Compared to traditional statistics, using machine learning algorithms increases prediction of euploidy in human embryos from weak (61%) to acceptable (72%)

S. De Gheselle[1], **J. Chambost**[2], **K. Declerck**[1], **C. Jacques**[2], **I. De Croo**[1], **C. Hickman**[2], **K. Tilleman**[1]

[1]*Ghent University Hospital, Department for Reproductive Medicine, Gent, Belgium ;* [2]*Apricity, Apricity, Paris, France*

Study question: To compare the ploidy prediction capability of traditional statistics versus machine learning when assessing human embryo morphokinetics.

Summary answer: Compared to traditional statistics, using deep learning algorithms increases prediction of euploidy in human embryos from 61% to 72%.

What is known already: Use of traditional statistics to predict PGT outcome using embryo morphokinetics has been attempted by various groups, with conflicting results as to whether it is possible to find significant morphokinetic markers to predict ploidy status of embryos. Conflicting results may be due to differences in approach. The main limitation is that regression analysis cannot exploit interconnections between predictors, whilst artificial intelligence approaches can. To our knowledge, this is the first attempt to compare the efficacy of prediction of traditional statistics with machine learning approaches in IVF. Moreover, there are limited studies using machine learning predictors to assess morphokinetic parameters.

Study design, size, duration: Traditional statistics and machine learning methods were used to assess the same retrospective dataset, (Jan2016-Dec2019) consisting of 445 biopsied embryos (129cycles) analysed for morphokinetic development using time-lapse imaging (EmbryoScope™). Trophectoderm biopsy was performed on day5 (D5) or 6 (D6). PGT for structural rearrangements or aneuploidy screening was performed by Next Generation Sequencing(NGS). The embryos were classified as either euploid [n=222 (178 D5, 44 D6)] or as displaying a chromosomal anomaly [n=223 (168 D5, 55 D6)].

Participants/materials, setting, methods: Input included morphokinetics (17 absolute and 7 time-interval timepoints), patient (such as age) and cycle (number of injected oocytes, 2pn fertilized, embryos biopsied) characteristics. Multiple logistic regression (MLR) analysis was performed on log-transformed morphokinetics parameters (SPSS) and on the entire dataset. For Machine learning; a random forest model (RFM) was used (80% of the dataset as training set and 20% as testing set). ROC analysis was performed to analyse the performance of the two approaches.

Main results and the role of chance: Using MLR (data displayed as geometric mean), only the following inputs were significantly different in embryos displaying a chromosomal numerical and/or structural anomaly versus embryos with an euploid profile respectively: t9+[72.61h versus 70.96h;p=0.007], tSC [82.60h versus 83.17h;p=0.05], tM [92.26h versus 92.47h;p=0.047], the time interval for the duration of compaction (tcomp) [8.32h versus 7.56h;p=0.043] and patient age (33.8 ± 4.6y vs 32.5 ± 4.0 years;p=0.031) achieving an AUC of 61% (CI: 56%-67%).

Using machine-learning, it was found that each absolute time point needed to be normalized by its previous morphokinetic parameter to reduce redundancy. The ranking of importance in predicting ploidy was tsC, t9+, t7, age, tM, t3, t6 and tPB2 (importance greater than 0.05). The following parameters approached significance (tB, tPNf, tSB, importance greater than 0.04). The remaining parameters, on their own, were not significantly predictive of ploidy. The top 7 parameters explained only 37% of the ploidy prediction, emphasizing the importance of using multiple datapoints for prediction of ploidy. This may explain discrepancies between previous papers in attempting to find a relationship between morphokinetics and ploidy.

Machine learning achieved an AUC of 72% (CI: 68%-77%) in the testing data set which was significantly higher than achieved with traditional statistics.

Limitations, reasons for caution: Dataset derived from a single clinic: generalization of results for other clinics was not assessed. Future work will include deep learning methodology on larger datasets with more diverse datapoints, using a data sharing hub, increasing data size, complexity and diversity to optimize the capabilities of machine learning in ploidy prediction.

Wider implications of the findings: Machine-learning raised the accuracy from 61% (weak) to 72% (acceptable) compared to MLR. Given the vast amount of variables impacting ART outcomes, traditional statistical methods should be replaced with machine learning and artificial intelligence approaches, becoming the new established standard for modelling prediction in ART.

Trial registration number: BC-07095

O-114 A universal algorithm is available in last generation time-lapse incubators: embryo score provided by the KIDScoreD5 is strongly correlated with chromosomal status and clinical outcomes.

L. Bori[1], F. Meseguer[1], M.A. Valera Cerdá[1], L. Alegre[2], A. Tejera[2], J. Remohí[3], M. Meseguer[2]

[1]IVIRMA, Research laboratory, Valencia, Spain ;
[2]IVIRMA, IVF laboratory, Valencia, Spain ;
[3]IVIRMA, Co-President, Valencia, Spain

Study question: Is the inbuilt software in EmbryoScope and EmbryoScopePlus systems useful to identify embryos with normal chromosomal status and high potential to achieve a live birth?
Summary answer: The embryo score provided by KIDScoreD5 algorithm is highly different depending on chromosomal status and the likelihood of achieving a pregnancy and a live birth.
What is known already: Time-lapse technology has allowed embryologists to develop selection algorithms with morphological and morphokinetic parameters. Numerous models have been described, but no one has yet been sufficiently consolidated for universal use. The EmbryoScope and EmbryoScopePlus systems include a selection method, KIDScoreD5, which classifies the embryos in categories based on cleavage time points and blastocyst appearance. The version 2 (v2) considers PN, t2, t3, t4, t5, tB and trophectoderm quality. Later, the Inner Cell Mass quality was added in version 3 (v3). To our knowledge, this is the first time that these newest versions are validated with such a large sample size.
Study design, size, duration: This retrospective analysis including 22,461 embryos from 2017 to 2019 was performed in IVI Valencia. Embryos were cultured in the time-lapse systems EmbryoScope and EmbryoScopePlus and routinely evaluated by senior embryologists according to the ASEBIR criteria. The EmbryoViewer software automatically detected morphological and morphokinetic parameters. If some error occurred, it was manually modified. Then, embryos were graded using the KIDScoreD5 algorithm in different scores from low to high quality (1-9.9).
Participants/materials, setting, methods: The KIDScoreD5 algorithm was tested with 7,857 embryos for v2 and 14,604 for v3. The embryo score was compared with the morphological grade assigned by embryologists, from A to D and excluded embryos. The correlation between the score of 3,311 embryos that underwent preimplantation genetic testing with their normal or abnormal chromosomal status was also studied. Finally, the association between the embryo score and clinical outcomes was analyzed in 3,296 Known Implantation Data (KID) embryos.
Main results and the role of chance: The comparison between the embryo score provided by the KIDScoreD5 and the category assigned by embryologists showed a direct association*. The means in V3 were 8.2 ± 1.2 for A; 5.7 ± 1.4 for B; 3.6 ± 1.2 for C, 2.2 ± 1.0 for D and 1.8 ± 0.7 for excluded embryos. Regarding the chromosomal status, embryos with normal content had significantly* higher score than abnormal ones. The score means and standard deviations for the newest version were 4.6 ± 1.8 for abnormal embryos and 5.3 ± 1.9 for normal ones. Embryos with higher marks achieved significantly* more implantation rate and live birth rate in both versions. Following results belong to V3 and are presented per quartiles of similar sample size. The implantation rates were 41.0% for score < 5.3, 54.2% for score 5.4-6.4, 59.3% for score 6.5-7.4 and 67.9% for score >7.5. The live birth rates were 20.2% for score < 5.3, 25.1% for score 5.4-6.4, 40.3% for score 6.5-7.4 and 48.6% for score >7.5. In addition, V3 was capable of distinguishing between implanted and non-implanted good quality blastocysts (A+B)*. The score means were 6.8 ± 1.5 for implanted good quality embryos and 6.4 ± 1.6 for non-implanted ones.
*p<.05
Limitations, reasons for caution: This project is limited by its retrospective and single-center nature. Multicenter validation would be necessary to corroborate the universal use of the KIDScoreD5 algorithm included in last generation time-lapse incubators.
Wider implications of the findings: This study showed the capability of the KIDScoreD5 in distinguishing between potential embryos with similar morphological characteristics. Therefore, embryo score could help embryologists to make decisions. Recently, time-lapse technology has taken a step forward towards automated annotations. The combination of universal selection models and automatism could improve the embryo selection.
Trial registration number: not applicable

O-115 Artificial Intelligence (AI) system combining both images and non-image inputs can improve the accuracy of human embryo viability prediction

I. Miyatsuka[1], K. Shimizu[1], T. Trisitichoke[1], A. My, Le[1], N. Enatsu[2], M. Inubushi[2]

[1]NextGeM Inc., Data science, Tokyo, Japan ;
[2]Hanabusa Women's Clinic, Reproductive Medicine, Kobe-Hyogo, Japan

Study question: Can an ensemble artificial intelligence (AI) system which uses both images and non-image features enhance the accuracy of embryos viability prediction?
Summary answer: Compared to conventional embryologist morphology assessments and image-based AI algorithms, predicting embryo viability using ensemble AI models yielded 30% and 4% improvement in accuracy respectively.
What is known already: Recent studies have demonstrated the ability of AI and computer vision to outperform traditional morphological assessments at predicting the likelihood of clinical pregnancy using images of human embryos. Existing algorithms, however, focus exclusively on using embryo images while neglect other non-image inputs such as patients inspection history and embryos hormonal profiles. An ensemble AI model which allows for mixed inputs can simultaneously evaluate both image and non-image data, thus enhancing the accuracy of identifying embryo viability by positive fetal heartbeat.
Study design, size, duration: A retrospective analysis using 19,342 static Day-5 blastocyst images with related inspection histories from 9,961 infertile patients undergoing IVF or ICSI treatment at Hanabusa Women's Clinic between January 2011 and August 2019 was conducted. Hormonal profiles are available for 1,358 embryos. Since the ensemble AI model requires both patients inspection histories and embryos hormonal profiles as inputs, development of such models was restricted to this subset of 1,358 embryos.
Participants/materials, setting, methods: All 19,342 blastocyst images are from single embryo transfer with known pregnancy outcomes. Images were converted into grayscale and rescaled into a resolution of 800x600 pixels. Two types of algorithms were evaluated: an image-only model and an ensemble model which combines deep learning algorithms for image inputs and machine-learning algorithms for non-image inputs. Due to limited sample size (1,358), predictive accuracy for the ensemble model was evaluated by the 10-fold cross-validation method.
Main results and the role of chance: Out of 19,342 Day-5 blastocysts (7,717 with fetal heartbeat), 1,358 embryos containing all the required image and non-image inputs are reserved for testing purpose. The remaining 17,984 images were split into training (~90%) and validation (~10%) sets for the development of an image-only AI model. The ensemble AI model, on the other hand, were trained and tested using only 1,358 embryos in the testing dataset.

Accuracy is used as the main measure to evaluate the performance of AI algorithms and defined as the percentage of both viable and non-viable embryos correctly identified by the AI models.

All blastocyst images were graded by experienced embryologists according to the Gardner scoring system. The prediction accuracy of the embryologist morphology assessment is calculated as the percentage of grade 3-6 embryos which resulted in positive pregnancy with fetal heartbeat and is set as the benchmark rate at 49.8%.

The accuracy rate achieved by the image-only AI model was 62.7%. The accuracy rate achieved by the ensemble AI model was 65.2%, representing an improvement in prediction accuracy of 4% when evaluating against image-only AI model and 30% when evaluating against the visual inspection method performed by embryologists.
Limitations, reasons for caution: Samples were collected from one clinic which can limit our results' reproducibility. The accuracy of the ensemble AI model is limited by the small number of embryos that we used. Non-image features in the current model do not include information related to male factor data and the embryo culture process.
Wider implications of the findings: Even with limited training dataset, our results indicate that we can improve the accuracy of predicting good-quality embryos by incorporating relevant non-image features. Future models may allow for embryo quality prediction at even earlier embryonic stages prior to syngamy.
Trial registration number: not applicable

O-116 Camera-agnostic self-annotating Artificial Intelligence (AI) system for blastocyst evaluation

M. VerMilyea[1,2], J.M.M. Hall[3,4], S. Diakiw[4], A. Johnston[3,4], T. Nguyen[4], M.A. Dakka[4], A. Lim[5], W. Quangkananurug[6], D. Perugini[4], A.P. Murphy[4], M. Perugini[4]

[1]Ovation Fertility, Laboratory, Austin, U.S.A. ;
[2]Texas Fertility Center, IVF Laboratory, Austin, U.S.A. ;
[3]Australia/Australian Research Council Centre of Excellence for Nanoscale BioPhotonics, The University of Adelaide, Adelaide, Australia ;
[4]Presagen, Life Whisperer, Adelaide, Australia ;
[5]Alpha Fertilty Centre, IVF Laboratory, Petaling Jaya, Malaysia ;
[6]Safe Fertility Center, IVF Laboratory, Bangkok, Thailand

Study question: Can computer vision image annotation techniques be used alongside machine learning to provide reliable blastocyst evaluation that is robust to different camera or microscope types?
Summary answer: AI that combines automated embryo annotation, trained on optical microscope images alone, generalises to yield high accuracy and consistency for time-lapse derived images.
What is known already: Recent studies have shown that AI and computer vision can improve embryo selection and accurately predict clinical pregnancy from images of human embryos at a fixed time point (e.g. Day 5). These results are expanded to consider techniques that are robust to camera and microscope type, and objective focal length, including snapshots taken from cameras used in time-lapse incubators. Computer vision detection and segmentation techniques are able to improve the distribution of AI ranking scores, showing consistent accuracy when using EmbryoScope or (preliminary) GERI time-lapse incubator data, with only 2.2% sample deviation of accuracy across six different focal lengths.
Study design, size, duration: The original Life Whisperer model (VerMilyea et al, 2020), was retrained using extensive image augmentation, 2,530 non-time-lapse incubator microscope images of Day 5 blastocyst embryos, and related clinical pregnancy outcomes, from four US laboratories, two Australian laboratories and one New Zealand laboratory. The AI includes embryo-detection and segmentation to maximize generalizability across different imaging modalities. The AI was applied to double-blind datasets of optical microscope, EmbryoScope (Malaysia/Thailand) and GERI images from the US.
Participants/materials, setting, methods: 3,470 separate optical microscope, 221 EmbryoScope, and 38 GERI images from patients undergoing fertility treatment at 12 IVF laboratories in five countries were used to train, validate and test the AI accuracy, distribution, robustness to camera/microscope type, and objective focal length. Only images of Day 5 blastocysts for which pregnancy (heartbeat at first scan) outcome was known, were used. This study was determined exempt from IRB review by Sterling IRB, USA (#6467).
Main results and the role of chance: This is the first study to show that AI trained on standard Day 5 microscope images can generalize to time-lapse incubator images, demonstrating the robust and camera agnostic nature of this approach.

The AI accuracy for prediction of clinical pregnancy (fetal heartbeat) was 65.4% when averaged over a blind test set of Day 5 blastocyst images from 10 IVF clinics in four countries. The sensitivity of the AI was 86.2%, while the specificity varied depending on the curation of the dataset. New blinded datasets of Day 5 single images of blastocyst stage embryos from the EmbryoScope and GERI time-lapse systems were then assessed with the AI. The AI generalized well to the time-lapse derived images and consistent overall accuracy (59.1%) was achieved, with a sensitivity of 77.9%. Multiple focal lengths were also considered and showed only a 2.2% deviation of accuracy. Together these results suggest that this method of pre-processing and automated annotation, as well as AI trained on a globally diverse dataset, creates a generalizable AI that is robust to camera type and focal setting.
Limitations, reasons for caution: The GERI data set is small and therefore analysis of the distribution of scores from this set should be expanded. Additional consideration of camera effects and focal lengths from both EmbryoScope and GERI devices across a wider range of clinics should also be considered.
Wider implications of the findings: Constructing AI that is robust to image variation represents an advancement in the computer vision field. Applying these techniques to blastocyst-stage embryos demonstrates that AI can be robust and generalizable to different clinical environments. This suggests that AI in the clinical embryology setting is practical and scalable, regardless of hardware.
Trial registration number: Not Applicable

O-117 Automated evaluation system based on artificial intelligence and visualization technology can effectively improve the accuracy of blastocyst evaluation

S. Wang[1], L. Chen[1], C. Zhou[2], D. Zhang[2], H. Sun[1]

[1]The Affiliated Drumtower Hospital, Reproductive Medicine Center, Nanjing-Jiangsu, China ;
[2]GrowthEngine Information Technology Co.- Ltd., artificial intelligence laboratory, Beijing, China

Study question: In deep learning based human blastocyst image classification, where are the essential features from?
Summary answer: In deep learning based human blastocyst image classification, the essential features are from trophectoderm(TE) , inner cell mass(ICM) and zona pellucida(ZP).
What is known already: Deep learning is a kind of algorithms that use artificial neural networks as a framework to learn features from data. Deep convolutional neural networks have rapidly become dominant in medical image automatic analysis. There have been related automatic grading studies in the field of reproduction, but features used in deep leaning based medical image classification basis have not been explained.
Study design, size, duration: This study retrospectively analyzed the image of 1025 blastocysts from March 2017 to August 2018 at the center for Reproductive Medicine of Affiliated Drum Tower Hospital of Nanjing University Medical School in China.By mapping different grading to good quality blastocysts(graded 4 or higher,ICM or TE is not C)and poor quality blastocysts(graded less than 4,or ICM or TE is C).We randomly divided the images into training(80%), validation(10%) and test(10%) set.
Participants/materials, setting, methods: 10957 images of blastocysts were obtained by excluding images of incomplete or fuzzy embryos.The embryo classification model based on the convolutional neural network VGG-16 was on the training set based on transfer learning. Receiver operating characteristic (ROC) Curve and Area Under Curve (AUC) are used as performance measure of the classification model. Grad-cam algorithm is used to visualize the classification features of the model, and it is displayed in the form of thermal diagram.
Main results and the role of chance: An independent test set of 1,104 images of blastocysts acquired at 116±1h after fertilization was used to evaluate the quality assessment model of blastocysts, which achieve an accuracy rate of 91.74%. The AUC of the image classification model of blastocyst can reach 0.970. Using Grad-cam algorithm to visualize all blastocyst stage embryo images on the test set, it was found that key features relied on by the classification model were on TE, ICM, and ZP.
Limitations, reasons for caution: In order to further verify the features relied on in image classification of the model, multi-center blastocyst images are needed.
Wider implications of the findings: Automatic evaluation of blastocyst images helps to reduce the variability of embryologist assessments. Using visualization techniques to explain classification features can help embryologists understand the principles and functions of deep learning, and to promote the development of deep learning technology in clinical application.
Trial registration number: H2019011

O-118 The migration speed of nucleolar precursor bodies in pronuclei is a predictor of human embryo development

T. Inoue[1,2], S. Taguchi[2], M. Uemura[3], K. Miyazaki[2], Y. Yamashita[2]

[1]Hyogo College of Medicine, Department of Emergency- Disaster and Critical Care Medicine, Nishinomiya- Hyogo, Japan ;
[2]Umeda Fertility Clinic, Department of Gynecology, Osaka, Japan ;
[3]Kobe Gakuin University, Faculty of Rehabilitation, Kobe, Japan

Study question: Does the migration speed of nucleolar precursor bodies (NPBs) in male and female pronuclei (mPN and fPN) affect human embryo development?
Summary answer: In the zygote, having potential to develop into a blastocyst/baby, the migration speed of NPBs was faster, which is a novel predictor of embryo development.
What is known already: NPBs are not considered as simple nucleolar components transmitted from an oocyte to an embryo, and they could participate in genome remodeling during embryo development. In the zygote, pericentromeric, and centromeric heterochromatin surrounds most NPBs in a ring-like shape. NPBs are essential only shortly after fertilization, suggesting that they

might actively participate in centromeric chromatin establishment. Several studies have shown that NPBs are dynamic, and the characteristic NPB pattern may change within a short time during the syngamy process on time-lapse imaging. However, the relationship between NPB migration speed and embryo development is unclear.

Study design, size, duration: After ICSI, migration of 262 NPBs from 47 zygotes (12 patients) were analyzed, and embryonic development was prospectively observed until blastocyst (blastocyst: *n*=25, arrest: *n*=22). The relationship between NPB migration and live birth was retrospectively analyzed from take-home-baby (*n*=10) and negative-clinical-pregnancy patients (*n*=15) in frozen-thawed single ICSI-derived blastocyst transfer cycles. The zygotes were cultured in a time-lapse incubator (Geri+; CO_2, 6%; O_2, 5% at 37°C and 80±20% humidity), and images were recorded every 5 minutes.

Participants/materials, setting, methods: The mPN and fPN were identified by appearance location in a zygote (fPN appearance: just below polar bodies). The central coordinates of mPN, fPN, and 2–5 NPBs/PN were measured by Kinovea (motion capture software). Their central coordinates were confirmed/revised every image and were decided. The migration distance of NPBs between two sequential images was calculated as the standard of central coordinates of PN. Thereafter, the migration speed of NPBs was calculated.

Main results and the role of chance: The migration speed of NPBs was significantly faster in the blastocyst developed group than in the arrested group (mPN: 4.61±1.26 vs. 3.37±0.87 μm/h, *P*<0.001, fPN: 4.06±1.15 vs. 3.34±0.97 μm/h, *P*=0.024). The migration speed of NPBs in mPN was correlated with that of NPBs in fPN (*r*=0.65, *P*<0.001). The timing of blastocyst formation was correlated with the migration speed of NPBs in mPN (*r*=−0.546, *P*<0.002) and had a correlation tendency with that of NPBs in fPN (*r*=−0.367, *P*=0.05). In the arrested group, 68.2% embryos arrested until day 3. In univariate logistic analysis, the blastulation was related to the migration speed of NPBs in mPN and fPN, tPNf, tPNf-tPNa, t2, t2-tPNf, t3-t2, t3-tPNf, t5-t4, and t5-tPNf. In multivariate logistic analysis, the factor associated with blastocyst development was the migration speed of NPBs in mPN (OR: 5.14, 95%CI: 1.20–21.90, *P*=0.027). The migration speed of NPBs in mPN in take-home-baby patients was significantly faster than that in negative-clinical-pregnancy patients (4.64±0.67 vs. 3.75±0.79 μm/h, *P*=0.008). In contrast, the speed in fPN was not significantly different between both groups (4.30±1.24 vs. 3.80±0.92 μm/h, *P*=0.254). The migration speed of NPBs in mPN was correlated with that of NPBs in fPN (*r*=0.603, *P*=0.001).

Limitations, reasons for caution: We could not analyze the migration of NPBs in the z-axis direction. When NPBs were large in number or drastically moved, NPB tracking could not be performed. Although our results did not completely explain the relationship between NPB migration and embryo development, the findings should help in elucidating the relationship.

Wider implications of the findings: The migration speed of NPBs is a novel predictor of blastocyst development. NPB migration speed may add clinical value for embryo selection, which may be associated with live birth, and consequently, the time to live birth could be shorter. The predictor could be an attractive marker for non-invasive embryo selection.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 32: WHICH ARE THE OPTIMAL OVARIAN STIMULATION PROTOCOL?

07 July 2020 Parallel 2 10:00 - 11:30

O-119 What is the optimal gonadotropin releasing hormone (GnRH) antagonist protocol during ovarian stimulation for assisted reproductive technologies (ART)? A pairwise and network meta-analysis

C. Venetis[1], A. Storr[2], S.J. Chua[3], R. Wang[4], B. Mol[4], E. Gravotta[5], S. Longobardi[6], X. Yin[7], T. D'Hooghe[8]

[1]University of New South Wales, Centre for Big Data Research in Health & School of Women's and Children's Health, Sydney, Australia ;
[2]Flinders Fertility, Department of Reproductive Medicine, Adelaide, Australia ;
[3]University Hospital Geelong, Barwon Health, Geelong, Australia ;
[4]Monash University, Department of Obstetrics and Gynaecology, Clayton, Australia ;
[5]Merck Serono S.p.A, Medical Affairs Fertility EMEA, Rome, Italy ;
[6]Merck KGaA, Global Clinical Development Fertility, Darmstadt, Germany ;
[7]EMD Serono- Inc, R&D Global Biostatistics- Epidemiology & Medical Writing, Billerica, U.S.A. ;
[8]Merck KGaA, Global Medical Affairs Fertility, Darmstadt, Germany

Study question: Which GnRH antagonist protocol maximises ongoing pregnancy rate (OPR) when considering the type of GnRH antagonist, the protocol of initiation, and use of pre-treatment or not?

Summary answer: In pairwise and network meta-analysis of combined studies with GnRH antagonists (ganirelix and/or cetrorelix), OPR was maximised without pre-treatment and after a fixed protocol.

What is known already: Several GnRH antagonist protocols are currently used for ovarian stimulation in the context of assisted reproductive technologies (ART). These are based on the use of the most commonly available GnRH antagonists (cetrorelix and ganirelix), in either a fixed or flexible modality and with or without pre-treatment with oral contraceptive pills (OCPs). However, the effectiveness and safety of the type of GnRH antagonist (cetrorelix or ganirelix), type of protocol (fixed day 5/6 or flexible protocol) and the type of pre-treatment (no pre-treatment [NP] or pre-treatment with OCPs, estrogens, progestins) have not been properly evaluated.

Study design, size, duration: A systematic literature search was performed on 8th December 2018 in MEDLINE, EMBASE, CENTRAL, SCOPUS and Web of Science, according to PRISMA guidelines. Qualitative and quantitative syntheses of all direct (pairwise) comparative data were performed using the random-effects model. A frequentist network meta-analysis was also performed to leverage the power of data from randomized studies that compared various GnRH antagonist protocols with the long agonist protocol, while checking whether the underlying methodological assumptions hold.

Participants/materials, setting, methods: Randomized controlled trials performed on women undergoing controlled ovarian stimulation (COS) for ART treatment that compared different GnRH antagonist protocols (direct data) or a GnRH antagonist protocol with a long GnRH agonist protocol (indirect data) were included. Studies with a day 2/3/4 fixed antagonist protocol were excluded. The effect size of choice for dichotomous outcomes was the relative risk (RR) and uncertainty around these estimates was expressed using 95% confidence intervals (CI).

Main results and the role of chance: The searches resulted in 7,042 studies. Seventy-two studies were included in the systematic review and sixty-nine in the network meta-analysis. Studies were of low-to-moderate quality. There was no difference in clinical pregnancy rates when comparing cetrorelix with ganirelix (RR: 0.99, 95% CI: 0.74 to 1.33; I2=0%; 2 RCTs; 258 participants; low-quality evidence). OPR was not reported for this comparison. Lower OPR was observed after a flexible protocol compared with the fixed day 5/6 protocol (RR: 0.74, 95% CI: 0.58 to 0.96, I2=0%; 5 RCTs; 703 participants; moderate quality evidence) and after OCP pre-treatment compared with NP (RR: 0.79, 95% CI: 0.64 to 0.97; I2=0%; 4 RCTs, n=1,244 participants; moderate quality evidence).

Network meta-analysis of 41 RCTs (n=8,009 women) showed that the use of the fixed-OCP protocol resulted in a significantly lower OPR compared with fixed-NP protocol (RR: 0.84, 95% CI: 0.71 to 0.99; moderate-quality evidence). A flexible-NP protocol may result in lower OPR compared with the fixed-NP protocol; however, this was not statistically significant (RR: 0.85, 95% CI: 0.72 to 1.00; moderate-quality evidence). By comparing all available protocols, the surface under the cumulative ranking suggested that the fixed-NP protocol is most likely to result in the highest OPR.

Limitations, reasons for caution: Limited data for some subgroups (pre-treatment with estrogens or progestins and type of ovarian response) and for live birth; heterogeneity in the criteria used for flexible protocols; limited data directly comparing cetrorelix with ganirelix and, therefore, limited evidence to consider them as equivalent in combined studies comparing GnRH antagonist protocols.

Wider implications of the findings: In the general population, the fixed day 5/6 antagonist protocol without any pre-treatment should be considered the

optimal antagonist protocol. More trials are required to compare the effectiveness of ganirelix and cetrorelix separately, and identify the optimal protocol in women with high or low response.
Trial registration number: Merck KGaA, Darmstadt, Germany

O-120 Influence of the duration of GnRH-antagonist ovarian-stimulation protocol on IVF outcomes in patients with anovulation, endometriosis, premature ovarian failure (POF) and idiopathic infertility.

J. Gómez[1], J.V. Martínez-Sanchis[2], I. Iniesta Mirón[2], P.J. Fernández-Colom[3], J.M. Rubio Rubio[4]

[1]Hospital Universitari i Politècnic La Fe, Laboratory of Human Assisted Reproduction, Valencia, Spain ;
[2]Hospital Universitari i Politècnic La Fe, Laboratory of Reproduction, Valencia, Spain ;
[3]Hospital Universitari i Politécnic La Fe, Laboratory of Reproduction, Valencia, Spain ;
[4]Hospital Universitari i Politecnic La Fe, Laboratory of Reproduction, Valencia, Spain

Study question: To evaluate the effect of the length of ovarian-stimulation protocol on oocyte maturation, fertilization, clinical pregnancy, miscarriage and live births in four different infertile pathologies.
Summary answer: Duration of GnRH-antagonist-ovarian-stimulation protocol had an effect on oocyte-maturation rate in all groups and also pregnancies, live-births and miscarriages in endometriosis and idiopathic infertility patients.
What is known already: In last years, Gonadotropin-Releasing Hormone (GnRH)-antagonist protocols have become in first choice for ovarian-stimulation in infertile women due to its shorter stimulation duration and lower associated risk to suffer an ovarian hyperstimulation syndrome (OHSS). It has been demonstrated that follicular growth, oocyte maturation and endometrial development are affected by the length of gonadotropin stimulation. Very short or long exposure to gonadotropins could have a detrimental effect on oocyte maturity stage and endometrial receptivity, leading to poor embryo development and lower pregnancy rates. There is a lack of studies which evaluate these correlations taking into account the type of female infertility.
Study design, size, duration: Retrospective study which included a total of 3589 ICSI-cycles from January 2015 to December 2019. All patients were stimulated by similar GnRH-antagonist stimulation protocol for different period of time depending on patient's characteristics. Patients were distributed in four groups according to two selection criteria: female infertility pathology (1.Anovulation (n=492); 2.Endometriosis (n=449); 3.POF (n=722); 4.Idiopathic (n=1926) and three different time slots of ovarian-stimulation (≤ 8 days; 9-10 days and ≥ 11 days) into each group.
Participants/materials, setting, methods: Mean age of all patients were 34.47 which ranged from 19 to 40 years old. GnRH-antagonist protocol (Orgalutrant or Cetrotide) was combined to recombinant Follicle-Stimulating Hormone (FSH: ranged 150-300 IU) and human Menopausal Gonadotrophin, (hMG: 6500 IU). Oocyte retrieval was scheduled 36 hours (h) following human Chorionic Gonadotropin (hCG) and inseminated by ICSI at 39-40 h. Embryo transfers were performed on day 2 or 3 taking into account good-scored embryos based on morphological parameters.
Main results and the role of chance: We found a significant increase in oocyte maturation rate in all studied groups with longer periods of ovarian stimulation (1.Anovulation (P-value: 0.016): ≤ 8 days: 72.22^{a}±19.51; 9-10 days: 75.22^{ab}±12.90; ≥ 11 days: 78,86^{b}±13,79; Endometriosis (P-value: 0.011): ≤ 8 days: 71.81^{a}±24.11; 9-10 days: 78.05^{b}±14.04; ≥ 11 days: 80,00^{b}±14.17; 3.POF (P-value: 0.049): ≤ 8 days: 75.18^{a}±28.46; 9-10 days: 75.18^{a}±15.33; ≥ 11 days: 79.64^{b}±11.82; 4.Idiophaty (P-value<0.001): ≤ 8 days: 72.91^{a}±10.53; 9-10 days: 76.37^{b}±6.50; ≥ 11 days: 78.94^{c}±7.48), regardless type of infertility. Fertilization rate was similar in all group and periods of stimulation. Clinical pregnancy rate showed significant differences in Endometriosis-group (2) reaching the highest value (37.12±4.52; P-value:0.013) when patients were stimulated for 9-10 days compared to ≤8 days: 17.86±5.50 and ≥ 11 days: 22.63±38.38. Similar trend was observed in pregnancy rate in POF-group-(3) whereas Anovulation-(1) and Idiopathic-(4)-groups showed a slight increase with shorter stimulation periods (≤8 days) but there were not significant differences. Live birth rate was significant higher (28.41±35.02; P-value:0.011) in Endometriosis-group-(2) when ovarian-stimulation period was 9-10 days whereas in Idiopathic-group-(4) was reached with time slots ≤8 days (27.94±27.16; P-value:0.006). Finally, miscarriage rate was statistically significant lower (14.00±34.24; P-value:0.049) in Idiopathic-group (4) with shorter stimulation periods (≤8 days).
Limitations, reasons for caution: Reduced number of ICSI-cycles in some of studied groups. Baseline levels of FSH and Estradiol (E2) hormones should be included.
Wider implications of the findings: To perform a prospective and randomize study including baseline levels of FSH and E2 in order to standardize the optimal time slot of GnRH-antagonist-stimulation for patients with different types of infertility.
Trial registration number: k

O-121 Comparison of different progestin regimens for pituitary suppression during ovarian stimulation for assisted reproductive technology, a systematic review

B. Ata[1], E. Turkgeldi[2], P. Alexandru[2], S. Guler Cekic[2], S. Yildiz[2]

[1]Koc University, Obstetrics & Gynecology, Istanbul, Turkey ;
[2]Koc University Hospital, Obstetrics and Gynecology, Istanbul, Turkey

Study question: Do progestins differ in preventing premature ovulation during ovarian stimulation for assisted reproductive technology?
Summary answer: All progestins seem to effectively prevent premature ovulation in ART cycles. Low-quality evidence suggests that progestins can effectively prevent premature ovulation in ART cycles.
What is known already: Progesterone secreted by corpus luteum in normal menstrual cycle supresses luteinizing hormone (LH) secretion from the pituitary during the luteal phase. Studies showed that progestins can also supress LH in freeze all assisted reproduction technology (ART) cycles. It is important to inhibit pituitary LH secretion before oocyte pick up and generally gonadotropin (GnRH) antagonists are used to inhibit the LH surge. Progestins are as effective as GnRH antagonists in supressing pituitary LH secretion.
Study design, size, duration: This is a a systematic review of studies comparing the effectiveness of progestins in preventing premature ovulation during ovarian stimulation for ART. We searched several electronic databases, trial registers, and websites from the date of inception until June 1, 2019 for studies, which compared; i) two different progestins or ii) two different doses of the same progestin for pituitary suppression in ART. Only studies published in English as a full text article were included.
Participants/materials, setting, methods: Five randomized trials and cohort studies involving a total of 2404 women., which compared; i) two different progestins or ii) two different doses of the same progestin were included. The primary outcome was live birth rate (LBR) per woman. Secondary outcomes were live birth or ongoing pregnancy (LB/OP) per woman and per embryo transfer (ET), ongoing pregnancy, clinical pregnancy, positive pregnancy test, numbers of oocytes and metaphase-two oocytes, duration of stimulation and gonadotropin consumption.
Main results and the role of chance: Medroxyprogesterone acetate (MPA), dydrogesterone (DYG) and micronized progesterone (MIP) were compared in combinations of two in these studies. None of the studies compared all three progestins together. Three studies compared two different progestins: MPA versus DYG, DYG versus MIP and MPA versus MIP; two studies compared two different dosages of the same progestin 4 versus 10 mg of MPA and 100 mg versus 200 mg of MIP. The primary outcome was not reported in most studies however there were no differences between progestins for secondary outcomes. All progestins seem to effectively prevent premature ovulation in ART cycles. Lower doses of the same progestins were also similarly effective with higher doses.
Limitations, reasons for caution: The presence of a limited number of trials/studies, most of which are not randomized nor accounts for every woman starting stimulation are drawbacks, preventing definitive conclusions on the subject.
Wider implications of the findings: Progestins in general can become a reasonable alternative to GnRH analogues in ART cycles when a fresh embryo transfer is not intended.
Trial registration number: not applicable

O-122 Superiority of cumulative live birth rates after GnRH antagonist cycles relates to ovarian response: a cycle-specific analysis of data from a Japanese national registry

S.C. Jwa[1], M. Takamura[1], A. Kuwahara[2], T. Kajihara[1], O. Ishihara[1]

[1]Saitama Medical University, Department of Obstetrics and Gynecology, Saitama, Japan ;
[2]Graduate School of Biomedical Sciences- Tokushima University, Department of Obstetrics and Gynecology, Tokushima, Japan

Study question: Are cumulative live birth rates (CLBRs) in different ovarian stimulation protocols for assisted reproductive technology (ART) similar in groups with different ovarian responses?
Summary answer: Mild ovarian stimulation (clomiphene citrate [CC] and letrozole) achieves similar or superior CLBRs to GnRH antagonist protocols with suboptimal egg retrieval cycles (< 10 oocytes).
What is known already: CLBR is regarded as the most relevant outcome of IVF and has a strong relationship with the number of oocytes retrieved. To maximize CLBRs per oocyte retrieval, GnRH antagonist protocols are adopted for normal to high responders. However, there have been few reports on selection of ovarian stimulation protocols for suboptimal or poor responders in terms of CLBR.
Study design, size, duration: This is a large retrospective cycle-specific analysis of data from the Japanese ART registry, which includes all ART treatment cycles in Japan. A total of 20,4675 fresh autologous cycles performed in 2014 and 2015 and subsequent 179,313 frozen embryo transfer cycles up to December 2016 were included in the analysis.
Participants/materials, setting, methods: The cycles analyzed were as follows: GnRH agonist (n=66,962), GnRH antagonist (n=56,027) and mild ovarian stimulation using CC alone (n=30,098), CC + gonadotropin (Gn) (n=41,991), letrozole alone (n=4,363), and letrozole + Gn (n=5,234). CLBR per oocyte retrieval was defined as at least one liveborn baby from one fresh and subsequent frozen cycles. Odds ratios (ORs) for cumulative live birth were calculated using generalized linear model.
Main results and the role of chance: The overall CLBR was 27.6%; the highest being with GnRH antagonist protocols (31.2%), followed by GnRH agonist protocols (30.1%), and mild ovarian stimulation protocols (22.6%–24.4%). Compared with GnRH antagonist protocol, all other ovarian stimulation protocols achieved significantly lower overall adjusted ORs for cumulative live birth per oocyte retrieved. Stratification by number of oocytes retrieved revealed that, among high responders (>15 oocytes), GnRH agonist protocols still had significantly less chance of achieving cumulative live birth than GnRH antagonist protocols (adjusted OR, 0.72, 95% CI, 0.68–0.77). Among normal responders (10–15 oocytes), adjusted ORs of CC + Gn and GnRH agonist protocols did not differ significantly from those of GnRH antagonist protocols (adjusted ORs of CC + Gn, 1.04, 95% CI, 0.95–1.14, adjusted ORs of GnRH agonist protocols, 0.96, 95% CI, 0.91–1.01). However, adjusted ORs of mild ovarian stimulation using CC alone (adjusted OR, 1.43, 95% CI, 1.32–1.54), CC + Gn (adjusted OR, 1.07, 95% CI, 1.02–1.12), letrozole (adjusted OR, 1.35, 95% CI, 1.14–1.61) and letrozole + Gn (adjusted OR, 1.43, 95% CI, 1.27–1.61) showed higher ORs than those of GnRH antagonist protocols in suboptimal (<10 oocytes) and poor (<5 oocytes) responders.
Limitations, reasons for caution: Because the registry provides anonymous cycle-specific data, we could not distinguish women who received multiple fresh cycles during the study period. Further, indications for selecting specific ovarian stimulation protocols vary across clinics, potentially causing residual confounding. The registry lacks important confounders such as parity and indicators of ovarian reserve.
Wider implications of the findings: Our results suggest that for patients expected suboptimal or poor ovarian response, mild ovarian stimulation using CC or letrozole may be an alternative option for selecting ovarian stimulation in ART to achieve similar or superior CLBRs to GnRH antagonist protocols.
Trial registration number: This study was supported by Health and Labour Sciences Research Grants.

O-123 Development and validation of a gonadotropin dose-selection model for individualized ovarian stimulation in IVF/ICSI: an individual participant data meta-analysis (IPD-MA).

N. Schouten[1], R. Wang[2], M. Eijkemans[1], B.W. Mol[2], H. Torrance[1], T. Van Tilborg[1], F. Broekmans[1], ORT iOS IPD-MA study group.(3)

[1]University Medical Center Utrecht, Department of Obstetrics and Gynaecology, Utrecht, The Netherlands ;
[2]Monash University, Department of Obstetrics and Gynaecology, Melbourne, Australia ;
[3]ORT iOS IPD-MA study group, , ; Bastu, Ercan, Acibadem University Faculty of Medicine ; Bergh, Christina, Gothenburg University ; D'Hooghe, Thomas, Merck Healthcare KGaA ; Friis Petersen, Jesper, North Zealand Hospital ; Jayaprakasan, Kannamannadiar, Royal Derby Hospital ; Khalaf, Yacoub, London Bridge Hospital ; Klinkert, Ellen, University Medical Center Groningen, The Netherlands ; La Marca, Antonio, University of Modena and Reggio Emilia ; Vuong, Lan Ngoc, University of Medicine and Pharmacy at Ho Chi Minh City ; Lapensée, Louise, Clinique ovo ; Lensen, Sarah, Melbourne ; Magnusson, Åsa, Gothenburg University ; Allegra, Adolfo, ANDROS Day Surgery Clinic ; Nyboe Andersen, Anders, Rigshospitalet ; Oudshoorn, Simone, University Medical Center Utrecht ; Popovic-Todorovic, Biljana, Universitair Ziekenhuis Brussel ; Youssef, Mohamed, Cairo University

Study question: Can a gonadotropin dose-selection model be developed and validated that will help guide ovarian stimulation by maximizing safety without negatively impacting pregnancy/live birth rates?
Summary answer: Development and validation of a gonadotropin dosing-selection model results in a moderately to fairly accurate prediction of treatment effectiveness and safety.
What is known already: Many studies addressed the effect of individualized gonadotropin dosing in IVF/ICSI using ovarian reserve tests (ORTs) including anti-Müllerian hormone (AMH), antral follicle count (AFC) and basal follicle-stimulating hormone (bFSH). A Cochrane review reported no evidence that individualized dosing is effective at increasing ongoing pregnancy/live birth rates (OPR/LB), but may reduce the incidence of ovarian hyperstimulation syndrome (OHSS). Individual patient data meta-analysis (IPD-MA) can be used to develop/validate a universal prediction model to help choose the optimal dose that will maximize safety without a negative impact on OPR/LB and reduce costs by preventing OHSS and use of ineffective high gonadotropin doses.
Study design, size, duration: For this IPD-MA, electronical databases were systematically searched for dosing randomized controlled trials (RCTs) in women undergoing IVF/ICSI. Women were categorized as predicted low, normal or high responders based on ORTs. RCTs comparing different ovarian stimulation doses (standard, adjusted or individualized) were included if the outcomes of interest were reported: OPR/LB and/or (interventions to prevent) OHSS. Eligibility assessment was conducted by two independent reviewers. Corresponding authors of eligible studies were invited to join this collaboration.
Participants/materials, setting, methods: After transfer of the IPD, data were cleaned and inconsistencies were resolved with the corresponding authors. Missing data were imputed 100 times with multiple imputation by chained equations for systematically and sporadically missing multilevel data. Models were developed with logistic regression. Predictor variables were selected after pooling and backwards selection (using $p < 0.3$) and by using background literature. Internal-external cross validation was used to assess prediction model performance.
Main results and the role of chance: A total of 14 RCTs reporting on 3,455 women were included: 762 had an OPR/LB and 171 had a safety event (severe or moderate OHSS or OHSS preventive interventions). Candidate predictor variables were age, body mass index (BMI), AFC, AMH, bFSH, parity, cycle length, duration of infertility, smoking, GnRH-agonist or GnRH-antagonist use and IVF/ICSI. For age and BMI, restricted cubic splines were used. Selected predictor variables for the chance of OPR/LB (the effectiveness prediction model) were age, BMI, AFC and IVF/ICSI. Age, AMH and bFSH were selected as predictors for safety (the safety prediction model). Starting gonadotropin dose (range 75-600 IU) was forced in both models. Internal-external cross validation resulted in 14 area under the receiver operating characteristic curve (AUC) values and confidence intervals (CIs) for the effectiveness prediction model and 10 AUCs and CIs for the safety prediction model. These AUCs were summarized in a meta-analysis forest plot. The resulting overall AUC for the effectiveness model was 0.60 (95% CI of 0.55-0.65), which can be classified as moderately accurate. The resulting overall AUC for the safety model was 0.71 (95% CI of 0.64-0.79), which can be classified as fairly accurate. Heterogeneity was low in both analyses: I^2 was 0%.
Limitations, reasons for caution: Two participating groups are in the process of data transfer/cleaning, therefore these preliminary results may not reflect the final results. In the following months, predictions from these models have to

be combined to develop the clinically adaptable gonadotropin dosing tool. We expect to present this dosing tool at ESHRE.
Wider implications of the findings: This tool may aid in choosing the optimal gonadotropin stimulation dose per individual by minimizing safety risks and maintaining effectiveness in terms of OPR/LB rates. This could potentially reduce treatment costs, by preventing prescription of high gonadotropin doses in low responders and preventing OHSS in high responders.
Trial registration number: CRD42019115489

O-124 Natural micronized progesterone versus a GnRH antagonist in egg-donation cycles. An extended experience.

J.C. Castillo[1], J. Guerrero[1], R. Delgado[2], B. Moliner[1], L. Lydia[1], J. Ten[1], A. Fuentes[1], A. Bernabeu[1], J. Llacer[1], R. Bernabeu[1]

[1]Instituto Bernabeu, Assisted Reproduction Unit, Alicante, Spain ; [2]ACCUNA, Assisted Reproduction Unit, Alicante, Spain

Study question: Is oral natural micronized progesterone equivalent compared to a GnRH antagonist with respect to the oocyte yield retrieved in egg-donation cycles?
Summary answer: Oral natural micronized progesterone is comparable to a GnRH antagonist in terms of total number of oocytes and mature oocytes (MII) retrieved in egg-donation cycles.
What is known already: Studies in own eggs IVF cycles shows that exogenous oral natural micronized progesterone is capable of blocking the LH surge without compromising oocyte competence. The strategy may be also suitable for egg donors (cost effective, patient friendly, oral administration). However, scarce and conflicting evidence is available with regards to its performance in the context of ovarian stimulation in egg donors. The aim of this study is to evaluate the efficacy of oral progesterone compared to a GnRH antagonist protocol in terms of oocyte yield at egg retrieval in a large sample of egg-donation cycles.
Study design, size, duration: Retrospective analysis of egg-donation cycles (n=1090) performed between January 2019 to December 2019 to assess the performance of oral natural micronized progesterone (Progeffik® 200 mg/d) versus cetrorelix (Cetrotide® 0.25 mg/day) in ovarian stimulation cycles triggered with triptoreline acetate (Decapeptyl® 0.2 mg). The clinical outcomes per embryo transfer in matched recipients (n=598) receiving a fresh embryo transfer were also assessed.
Participants/materials, setting, methods: All participants received urinary FSH (Fostipur® 150-225 IU/d s.c.). The oral progesterone group (n=583) concomitantly received 200 mg/d micronized progesterone per os. The GnRH antagonist group (n=507) received cetrorelix 0 25 mg/d beginning with a leading follicle of 14 mm. Triptorelin 0.2 mg induced the final follicular maturation. Egg collection was performed 36 hours after triggering. The main outcome was the total number of oocytes and the number of MII retrieved at egg retrieval.
Main results and the role of chance: Overall, baseline and cycle characteristics were similar between progesterone and antagonist groups with regards to age: 25.2 (SD 4.4) vs 25.6 (SD 4.3) years old, AFC: 18.1 (SD 6.4) vs 18.5 (SD 7.4), BMI: 22.4 (SD 2.6) vs 22.8 (SD 2.7) kg/m^2; total dose of gonadotropins: 2088 (SD 817) vs 2086 (SD 633) IU and duration of stimulation: 9.8 (SD 1.4) vs 9.9 (SD 1.3) days; respectively. The total number and MII of collected eggs were no different: 15.8 (SD 7.5) vs 15.2 (SD 7.6), p=0.9 and 13.4 (SD 6.9) vs 12.9 (SD 7.1), p=0.7 in the progesterone versus GnRH antagonist group, respectively. In matched recipients, mean number of MII assigned were 9.5 (SD 1.3) vs 9.9 (SD 1.7) coming from the progesterone vs antagonist group, respectively (p=0.009). Fertilization rate was 78% in progesterone versus 75% in the antagonist group (p=0.001). Biochemical pregnancy rate was 64 versus 65% (p=0.7); clinical pregnancy rate 55.9 versus 56% (p=0.9) and ongoing pregnancy rate 47.7 versus 48%, (p=0.9).
Limitations, reasons for caution: Retrospective design. Without a formal sample size, findings after fertilization should be interpreted with caution. Additionally, changes in the laboratory were introduced in second half 2019, when the vast majority of cases received progesterone for LH peak prevention. Prospective, preimplantation genetic testing for aneuploidies studies on the topic are warranted.
Wider implications of the findings: These data, from a retrospective study design, suggest that exogenous progesterone is capable to block the LH surge without compromising oocyte yield or competence. This cost/effective, patient-friendly, oral administration approach may provide conveniences for egg donors thus, facilitating egg donation programs.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 33: PREDICTORS. TECHNOLOGY AND PROCESSES IMPROVING OUTCOMES IN ANDROLOGY

07 July 2020 Parallel 3 10:00 - 11:30

O-125 Live Births With a Novel ROSI Technique Using Elongating Spermatids for Non Obstructive Azoospermia Patients: A European Cohort Study

R. Pabuccu[1], S. Sertyel[2], E. Pabuccu[1], K. Aydos[3], A.H. Haliloglu[4], D. Demirkıran[2], G. Keles[5], A. Tanaka[6]

[1]Ufuk University School of Medicine, Obstetrics and Gynecology, Ankara, Turkey ; [2]Centrum Clinic Assisted Reproduction Center, IVF and Andrology, Ankara, Turkey ; [3]Ankara University School of Medicine, Urology, Ankara, Turkey ; [4]Ufuk University School of Medicine, Urology, Ankara, Turkey ; [5]Centrum Clinic Assisted Reproduction Center, IVF Section, Ankara, Turkey ; [6]Saint Mother Hospital., IVF Unit, Fukuoka, Japan

Study question: Can elongating spermatids be used for novel round spermatid injection (ROSI) technique in non-obstructive azoospermia (NOA) patients when no sperms are retrieved during a micro testicular sperm extraction (mTESE) procedure?
Summary answer: A novel ROSI technique using elongating (Sb2) spermatids and electrical oocyte activation reveals promising reproductive outcomes for NOA cases with a failed mTESE
What is known already: NOA is one of the leading causes of male infertility and nearly one half of all mTESE procedures fail to reveal mature spermatozoa for ICSI. For these cases, previously different stages of spermatids have been proposed for ICSI, however, with unsatisfactory reproductive outcomes. Recently, healthy live births were reported following a novel ROSI technique without major adverse events (Tanaka et al., 2018). In brief, selection of spermatids after mTESE, followed by disruption of the cell membrane, separation of the nucleus and intra cytoplasmic injection of the nucleus into electrically-activated oocytes are the basic steps.
Study design, size, duration: A cohort study was performed in a private IVF center from February 2018-December 2019. After urology consultation, all NOA cases underwent mTESE procedure on the day of oocyte pick-up (OPU). Testicular tissue was examined to collect spermatozoa forms when available. Otherwise, elongating (Sb2) spermatids were investigated to be used for the novel ROSI technique. After standard incubation, cleavage stage embryos (day 3) or blastocysts were transferred in either a fresh or frozen-thaw cycle.
Participants/materials, setting, methods: Signed informed consent was obtained from all patients. After mTESE, testicular tissue was evaluated under an inverted microscope with contrast system to classify spermatids if no spermatozoa were seen. After selecting Sb2 forms, elongated spermatid injection was applied, using the novel technology previously described for ROSI. After enzymatic digestion of the testicular tissue, spermatids were isolated, cell membranes were disrupted and finally the nucleus with the remaining cytoplasmic part was injected into electrically-activated oocytes.
Main results and the role of chance: A total of 472 couples underwent 904 cycles using elongating (Sb2) spermatids for the ROSI technique. Cases with the retrieval of mature spermatozoa and elongated spermatids were excluded from the present analysis. The mean age of women and men were 33.5±5 and 37±5, respectively. The mean number of oocytes was 6.6±4. The fertilization and cleavage rates were 49.9% (3024/6052) and 42.5% (2577/6052) respectively. The blastulation rate was 38.9 % (1005/2577). The number of cycles reaching a transfer was 78%. The number of positive hCG tests per ET was 14.2 % (102/714) and the number of ongoing pregnancies beyond 20 weeks was 9.6 % (69/714). A total of 8 live births have been recorded until now from this series, and pregnancies are still undergoing. The total live birth rate from the series will be reported during the conference.

Limitations, reasons for caution: The selection of early spermatogenic cells demands expertise as these cells could be indistinguishable from other round shape cells. Moreover, these cells do not properly trigger oocyte activation, thus electrical stimulation is needed for fertilization. Although early spermatogenic cells are haploid, genetic as well as epigenetic alterations might be possible.
Wider implications of the findings: The novel ROSI technique enables a proportion of males, for whom sperm donation or adoption until now was the only option, to father their own offspring. The present results support that elongating spermatids as an advanced form of round spermatids can be used for ICSI, resulting in healthy offspring.
Trial registration number: not applicable

O-126 The added value of enzymatic digestion to mechanical mincing in testicular sperm retrieval in non-obstructive azoospermia

V. Vloeberghs[1], A. Racca[1], B. Popovic-Todorovic[1], I. Mateizel[2], G. Verheyen[2], H. Tournaye[1]

[1]UZ Brussel, Obstetrics-Gynaecology, Jette- Brussels, Belgium ;
[2]UZ Brussel, Centre for Reproductive Medicine, Jette- Brussels, Belgium

Study question: To evaluate the added benefit of enzymatic digestionto mechanical mincing in testicular sperm retrieval for patients with non-obstructive azoospermia (NOA).
Summary answer: Enzymatic digestion increases the sperm retrieval from testicular biopsies of NOA patients by 32.3%.
What is known already: Although there is increasing evidence that the surgical technique to recover testicular sperm in NOA makes little difference as long as multiple biopsies are taken, the debate on which is the best technique continues. Few studies focus on the role of the lab and even fewer evaluated the benefit of enzymatic tissue digestion in order to improve sperm retrieval rates. Research in this domain has been flawed by heterogeneity of either the NOA diagnosis or the selected patient study population.
Study design, size, duration: A retrospective single-centre cohort study from 2004 till 2017 included all patients who underwent their first diagnostic or therapeutic testicular sperm extraction (TESE) by open biopsy. They all had normal standard genetic workup and subsequent histological confirmation of NOA. Patients who had a history of previous TESE or histological diagnosis of hypospermatogenesis were excluded. Primary outcome was sperm retrieval for either cryopreservation or intracytoplasmic sperm injection (ICSI).
Participants/materials, setting, methods: Up to 6 biopsies were obtained from each testis. In cases with no or insufficient number/quality spermatozoa observed during 30 min after mechanical mincing, enzymatic treatment of residual tissue pieces was performed with collagenase type IV. The results were divided according to the biopsy type (diagnostic or therapeutic). In order to evaluate which factors predicted sperm retrieval, multivariable regression analysis was performed adjusting for the following confounding factors: age, FSH level, testicular volume and histology.
Main results and the role of chance: In total, 425 patients were included. Overall, in 69/425 (16.2%) men, spermatozoa for cryopreservation or ICSI were found after mechanical mincing. In 115/356 (32.3%) patients who had no sperm following mincing, spermatozoa were found after enzymatic digestion. In order to reduce bias in sperm retrieval rate when TESE is scheduled on the day of ICSI (therapeutic) or prior to planning ICSI (diagnostic), these two patient populations were analyzed:316 (74.4%) in the diagnostic and 109 (25.6%) in therapeutic testicular biopsy group. There was no difference in mean age (34.9 vs. 35.9 years), mean volume of testicles (10.9 vs. 10.2 ml), histological diagnosis (Sertoli Cell only 56.1 vs. 56%; maturation arrest 21.8 vs. 21.2%; sclerosis and/or atrophy 19.9 vs. 18.4%) between the diagnostic vs. therapeutic group. FSH was significantly higher in the diagnostic group compared to the therapeutic group (22.3 vs. 18.5 IU/l, p=0.008). Sperm retrieval was significantly higher in the therapeutic vs. diagnostic group both after mincing (24.8% vs. 13.9%, p=0.007) and enzymatic digestion (43.1% vs. 21.5%, p< 0.001%). Multivariable logistic regression analysis showed that enzymatic digestion remained a significant predictor of sperm retrieval, when adjusting for potential confounders.
Limitations, reasons for caution: The main limitation of the study is its inherent retrospective design.
Wider implications of the findings: Enzymatic digestion in addition to mechanical mincing increases sperm retrieval substantially in NOA patients following either a diagnostic or a therapeutic TESE.
Trial registration number: Not Applicable

O-127 Ooplasm-Mediated Sperm Nuclear Decondensation for Heritable Genome Editing of the Mammalian Male Gamete

M.S. Wang[1], P. Xie[1], A. Trout[1], Z. Rosenwaks[1], G. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Can we identify a method to access the DNA of spermatozoa and allow gene editing by CRISPR-Cas9?
Summary answer: An ooplasm-mediated approach is effective in allowing sperm decondensation for CRISPR access and successful genome editing in the mouse.
What is known already: Previous studies on heritable genome editing (HGE) have been carried out at the zygote phase; however, they faced the difficulties of mosaicism and embryo research ethics. These issues may be resolved by first editing the DNA of the gametes. The challenge with spermatozoa, however, resides largely in their distinct structure—they contain tightly packed chromatin, carry no DNA repair mechanisms, and are highly susceptible to damage. Our previous attempts to directly edit spermatozoa DNA required considerable resources but yielded limited results. We hypothesize that an alternative cellular environment may be necessary to allow proper male genome editing.
Study design, size, duration: Over a period of 3 months, 88 oocytes were divided into two groups. One group was used for ooplasm-mediated sperm decondensation (OMSD) to produce haploid embryos containing only DNA from the male. The control group consisted of embryos produced through standard ICSI, following the established HGE approach. Both groups were treated with a CRISPR solution designed to knock out Tyr to create an albino phenotype. Gene editing success was then compared between the groups.
Participants/materials, setting, methods: Oocytes and sperm were collected from B6D2F1 mice. OMSD oocytes were enucleated and allowed 2 hours to rest. All oocytes then underwent ICSI with the addition of a CRISPR-Cas9 solution containing pre-complexed Cas9 protein and Tyr knockout gRNA. Embryos were incubated for 24 hours, and then cryopreserved. Later, DNA was extracted and amplified for T7E1 analysis of the target site.
Main results and the role of chance: Of the 88 oocytes, 31 were enucleated for the OMSD condition, while 57 constituted the control. After undergoing ICSI with CRISPR solution, 74% (23/31) of the OMSD oocytes survived compared to 75% (43/57) of the control. Of those, 43% (10/23) of the experimental group developed to the 2-cell stage, while 70% (30/43) of the control progressed (p<0.05). After extraction, a 423-bp region around the CRISPR target site was amplified. A total of 35 embryos produced a sufficient concentration of DNA, while 3 OMSD and 2 control embryos failed to amplify. Gene modification at the target site was confirmed in 29% (2/7) of OMSD embryos and 36% (10/28) of the control group. We achieved successful genome editing of spermatozoa DNA through the use of ooplasm-mediated sperm decondensation at a comparable rate to zygotic heritable genome editing. It is important to note that these results are likely to be underestimated, since any embryos with completely uniform modifications may not be detected by T7E1.
Limitations, reasons for caution: Currently, sample size is limited. Refining of experimental techniques is needed to improve survival and development rates, and DNA sequencing of blastomeres will provide a more accurate evaluation of editing efficiency. Additionally, off-target effects, mosaicism, epigenetic modifications, and birth rate have yet to be evaluated.
Wider implications of the findings: Once editing efficiency is optimized, individual pseudo-blastomeres of OMSD embryos can be used in place of spermatozoa to fertilize oocytes. Subsequently, the pre- and post-implantation development of offspring that contain the intended genetic modifications can be studied. Our model, if reproduced in human, may prevent the inheritance of paternal mutations.
Trial registration number: not applicable

O-128 A Novel Microfluidics Method for Reliable and Efficient Sperm Sex Selection

R. Elias[1], S. Cheung[1], A. Parrella[1], P. Xie[1], D. Keating[1], Z. Rosenwaks[1], G. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Can selection of Y-specific spermatozoa be achieved through ATP content modulation in a microfluidics system?

Summary answer: Ligand activation on the sperm flagellum decreased motility of the female spermatozoa and ensured expedited Y-bearing spermatozoa selection in a microfluidics chamber.

What is known already: Several techniques have been proposed to successfully select for X- or Y-bearing spermatozoa, such as centrifugation methods, layering techniques, electrophoresis, and flow cytometry. At our clinic, we have been able to carry out sex selection with an 80% success rate with the use of a proprietary density gradient technique that requires over 3 hours of processing. However, it has recently been proposed that modulation of the Toll-like receptor 7/8 (TLR7/8) on the X-bearing sperm flagellum impairs motility and may therefore be effective in sex selection techniques.

Study design, size, duration: In the last 5 years, we processed ejaculates from 98 consenting couples undergoing treatment at our center with a custom multilayer density gradient. The proportion of X- and Y-bearing spermatozoa before and after selection was assessed by fluorescent in situ hybridization (FISH). Cycle pregnancy outcomes and offspring sex were recorded. To expedite our selection process while maintaining sex enrichment, we tested a new method utilizing TLR7/8 ligand activation together with microfluidics.

Participants/materials, setting, methods: A total of 98 couples were treated at our center (IRB 1306014043) in 120 cycles. A proprietary multilayer density gradient method was used to select for sex-specific spermatozoa. We confirmed successful selection by FISH analysis on at least 1,000 cells/specimen. Pregnancy outcomes and offspring sex were assessed and compared. In a new selection technique, we incubated spermatozoa for 45 minutes in HTF medium containing 0.3μM of the TLR7/8 ligand, prior to microfluidics processing.

Main results and the role of chance: Of the couples (maternal age, 36.8±4yrs; paternal age, 39.6±5yrs) included, 53.1% wanted a female child, while 46.9% desired a male child. The initial sperm concentration was 65.4±26x10^6/ml, with 47.7±5% motility, normal morphology, and average sperm aneuploidy of 3.3±4%. After multilayer density gradient selection, sperm concentration decreased to 24.3±14x10^6/ml, while motility rose to 94.5±3% (P<0.0001).

Of the 52 couples who wanted a female child, FISH assessment confirmed a spermatozoa enrichment of 80%, and over 80% of couples obtained a female embryo as assessed by PGT-A. The clinical pregnancy rate for these couples was 25.7% (18/70), with a delivery rate of 61.1% (11/18) of the desired sex; the remaining pregnancies are ongoing.

For 46 couples who desired male offspring, FISH analysis confirmed a successful enrichment for Y-bearing spermatozoa, and over 80% of couples obtained a male embryo. The clinical pregnancy rate for these couples was 28.0% (14/50), and the delivery rate was 57.1% (8/14) of the desired sex; the remaining pregnancies are ongoing. To test our new method, in a separate non-clinical investigation, four additional sperm specimens were processed by the proposed ligand activation technique, yielding >80% enrichment, confirmed by FISH, that was comparable to the more time-consuming multilayer density gradient technique.

Limitations, reasons for caution: Although the TLR7/8 ligand mechanism is not absolutely clear, its effect on the flagellum is reversible and therefore does not affect acrosomal function or viability. Nevertheless, the capacity of spermatozoa selected by this technique to support embryonic development needs further investigation.

Wider implications of the findings: We were able to enhance the proportion of female and male embryos with our current multilayer density gradient technique requiring >3 hours. However, the proposed method incorporating ligand activation and microfluidics would lower this processing time to <1 hour. We are refining this method in order to achieve >80% selection.

Trial registration number: not applicable

O-129 Effect of microfluidic sperm separation versus standard sperm washing processes on fertilization rates, blastocyst development and euploidy rates among all infertility patients

G. Adaniya, E.L.D.- H.C.L.D.- Ph[1], C. Leisinger[2], M. Freeman[3], E. Behnke[4], M. Aguirre[5], T. VerMilyea[6], M. Schiewe[7]

[1]Ovation Fertility, IVF Laboratory, Carmel- IN, U.S.A. ;
[2]Ovation Fertility, IVF Laboratory, Baton Rouge- LA, U.S.A. ;
[3]Ovation Fertility, IVF Laboratory, Nashville- TN, U.S.A. ;
[4]Ovation Fertility, IVF Laboratory, Cincinnati- OH, U.S.A. ;
[5]Ovation Fertility, IVF Laboratory, Las Vegas- NV, U.S.A. ;
[6]Ovation Fertility, IVF Laboratory, Austin- TX, U.S.A. ;
[7]Ovation Fertility, IVF Laboratory, Newport Beach- CA, U.S.A.

Study question: Our goal was to determine if the ZyMōt microfluidic sperm separation device effectively improved ICSI sperm selection and subsequent embryo development applied to a general IVF patient population.

Summary answer: Utilization of a ZyMōt device to process all IVF semen samples (untested for Sperm DNA Fragmentation, SDF) revealed no overall benefit to embryo outcomes.

What is known already: The natural sperm filtering actions of cervical/uterine crypts has been mimicked by a new microfluidic sperm separation device (ZyMōt). Its microporous filter and channels help separate the most motile sperm with normal morphology without centrifugation. In turn, not only does the ZyMōt device claim to reduce procedure-induced reactive oxygen species (ROS) associated with causing SDF, it selectively isolates healthier, progressively motile sperm with lower SDF. Pilot studies suggest that the higher chromatin integrity (i.e., lower SDF) attained for IVF use improves fertilization, embryo morphology and blastocyst euploidy rates. We aimed to test the ZyMōt's proposed developmental benefits.

Study design, size, duration: A prospective, randomized, multicenter, sibling oocyte study was conducted with 86 consenting patients possessing ≥10 oocytes. Non-DFI tested ejaculates underwent a split processing treatment: 1) Control washing procedures (density gradient separation or swim-up) or 2) Treatment - ZyMōt swim out. Each sample was then used to inseminate an equal number of sibling oocytes. Resulting blastocyst were biopsied and NGS tested. Euploid (non-mosaic) embryo selection for ET was randomized. Chi-squared analysis was performed to assess differences($p<0.05$).

Participants/materials, setting, methods: Upon informed consent, partner sperm was processed in parallel by 1) either density gradient centrifugation (DGC) at OF-IN(n=26), OF-OH(n=19) and OF-LA(n=18) or a swim-up wash procedure (OF-TN,n=23); or 2) a 0.85μ ZyMōt device. After removal of cumulus/coronal cells by hyaluronidase, half of the mature oocytes were randomly allocated to ICSI with Control sperm and the other half with ZyMōt sperm. Fertilization, embryo culture and biopsy were performed using the clinic's standard protocols.

Main results and the role of chance:

Table. ICSI cycle embryo development outcomes following standard sperm washing procedures (Control) or the use of a microfluidic sperm separation device (ZyMōt)

Treatment:	ZyMōt	Control
Number of Oocytes	787	777
Fertilization rate (% 2PN)	604 (76.7%)	592 (76.2%)
Blastocyst rate (%>2BB)	296 (49.0%)	282 (47.6%)
Euploidy rate	165/283 (58.3%)	151/265 (57.0%)
Mosaicism rate	25/283 (8.8%)	31/265 (11.7%)

There were no statistical differences observed between the ZyMōt or Control sperm processing methods. Although some normal variation between labs was observed, there were also no differences between the control DGC and swim-up methods. Clinical pregnancy outcomes are still being evaluated, but preliminary results reveal no advantage to the ZyMōt treatment. To date, intrauterine

pregnancies are trending upward(p>0.1) for the Control groups (21 of 30, 70%) in contrast to 18 of 37 (48.6%) in the ZyMōt group.
Limitations, reasons for caution: Male factor patients with known elevated SDF were not selected for this multi-center trial. Therefore, we can not speak to the potential improvements ZyMōt may elicit. However, it is possible that selection of normal, progressive sperm for ICSI, plus the repair capacity of oocytes, is sufficient to promote normal development.
Wider implications of the findings: We do believe the ZyMōt device may have a useful application in infertile men with elevated SDF, yet it is equally possible that an ICSI sperm selection alone allows for the immobilization and injection of highly progressive, morphologically normal sperm as seen in this study of typical infertility patients.
Trial registration number: OF191218B

O-130 Testicular cell communication mediated by extracellular vesicles

E. Fok[1], K.H.K. Choy[1], S.Y. Chan[1], W. Lam[1]

[1]The Chinese University of Hong Kong, School of Biomedical Sciences- Faculty of Medicine, Hong Kong, Hong Kong

Study question: Is extracellular vesicle (EV) presence in the mouse testis? If yes, do these testicular EVs mediate cell communication in mouse testis?
Summary answer: EVs were present in mouse testis. Testicular EVs were uptake by both germ cells and somatic cells and they carry novel small RNA cargos.
What is known already: Spermatogenesis requires a precisely orchestrated cellular communication networks. EV is known to be ubiquitously released by eukaryotic cells. EV carries various cargo, including genomic DNA, mRNA, miRNA and proteins, that can be uptake by target cells and regulate their cellular processes, indicating that EV is an important and effective way of cell-cell communication. EVs secreted by the prostate and the epididymis have been characterized and shown to play important roles in sperm maturation and functions. However, the presence of EVs in the testis and their involvement in spermatogenesis remain elusive.
Study design, size, duration: We collected testis samples from mouse and isolated testicular EVs using a newly established tissue dissociation method.
Participants/materials, setting, methods: The physical properties of testicular EVs were assessed by dynamic light scattering and electron microscopy. Testicular EVs were validated by Western blot of established EV markers and Golgi marker. The small RNA cargo was profiled by RNA-sequencing and verified by real-time PCR. The uptake of testicular EVs was examined by the fluorescence-labelling of EVs.
Main results and the role of chance: Testicular EVs were isolated from the mouse testis using the newly established tissue dissociation method. Electronic microscopy results showed the hallmark cup-shape morphology of EVs with sizes ranged from 50 – 200 nm. The presence of hallmark EV markers CD63, CD81 and CD9 and the negligible amount of Golgi marker suggest clean isolation of testicular EVs with minimal membranous organelle contamination. Small RNA sequencing (RNA-seq) revealed RNA species commonly observed in EVs, including rRNA, tRFs, snoRNA, miRNAs and piRNAs. Importantly, we have identified three novel miRNAs that were not reported in the testis transcriptome. To study the type of testicular cells that uptake testicular EVs, we injected PKH-labelled testicular EVs into the interstitial space or seminiferous tubules. The results showed that EVs were uptake by Sertoli cells and all stages of germ cells.
Limitations, reasons for caution: The physiological function of testicular EVs awaits further investigation.
Wider implications of the findings: The testicular EVs may provide novel insight into our understanding of sperm production and inheritance.
Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 34: ENDOMETRIOSIS - PATHOGENESIS AND DIAGNOSIS

07 July 2020 Parallel 4 10:00 - 11:30

O-131 Exome-wide screen for somatic mutations in endometriosis lesions

K. Ward[1], R. Chettier[2], H.M. Albertsen[2]

[1]Juneau Biosciences- LLC, Administration, Salt Lake City, U.S.A. ;
[2]Juneau Biosciences, Research and Development, Salt Lake City, U.S.A.

Study question: Which genes undergo somatic mutation during the development and progression of endometriosis lesions; do these genes differ from those that predispose women to develop endometriosis?
Summary answer: Endometriosis lesions develop somatic mutations in several genes. The mutations identified in lesions differ from germline variants that predispose women to develop endometriosis.
What is known already: Endometriosis is a clinically heterogenous condition affecting 10% of women. It is well established that germ-line mutations may predispose some women to develop endometriosis, however penetrance, expressivity, and pleiotropy of the "initiating" genes implicated to date are not understood. Clinical heterogeneity is even observed within a single patient as an affected woman may have some lesions that are progressive, invasive, and possibly metastatic or malignant while other lesions are not. Recent studies have shown that somatic mutations accumulate during the clonal evolution of individual endometriosis lesions.
Study design, size, duration: This study is a retrospective cohort association study. 2,932 DNA samples from unrelated women with surgically-confirmed endometriosis (GERMLINE) and 274 tissue blocks containing endometriosis lesions (LESION) underwent whole exome sequencing. Fisher's Exact Test was used to determine excess burden (alpha <0.001).
Participants/materials, setting, methods: DNA was extracted using standard methods. Exome sequencing was performed using an Ion Proton Instrument, AmpliSeq Exome Capture, and the Torrent Variant Caller. Missense and truncating variants having a minor allele frequency <0.005 in the gnomAD database were considered for analysis. We calculated somatic burden for each gene by first counting protein-altering variants identified in only one patient per group. We also calculated cumulative genetic burden for each gene by adding all coding variants identified.
Main results and the role of chance: A mean of 63 "singleton" variants per patient were detected in the GERMLINE samples and 65 singleton variants per patient in the LESION group. 11 genes showed excess somatic mutations in the LESION samples when compared to the GERMLINE results (MIEF1, MSX1, FAM149A, VEZT, FAM160B1, PCDHA6, RIBC1, IRAK4, HYAL2, AQP4, and DSE). Singleton variants in these genes were observed at a 6.3 to 22 fold excess in the LESION samples when compared with the expectations from the GERMLINE samples. None of these genes are among the top 1000 FLAGs ranking (frequently mutated genes). VEZT has been previously implicated as an endometriosis associated gene through GWAS studies.
Limitations, reasons for caution: The sample size is modest and replication of these results in additional cohorts is warranted. It is very unlikely formalin fixation or paraffin embedding caused the observed mutations, but untreated LESION samples are currently being tested in our laboratory.
Wider implications of the findings: Discovery of gene mutations underlying endometriosis progression may lead to new pathophysiology insights, improved diagnostics, and novel treatment approaches.
Trial registration number: Not applicable

O-132 The landscape of endometrial transcriptome in ovulatory obese PCOS women

V. Salamun[1,2], E. Vrtačnik Bokal[1], B. Peterlin[3], A. Maver[3], N. Kočevar[3], O. Maloku[4], T. Papler Burnik[1]

[1]University Medical Centre Ljubljana, Department of Human Reproduction-Division of Obstetrics and Gynecology- University Medical Centre Ljubljana-Ljubljana- Slovenia, Ljubljana, Slovenia ;
[2]Medical faculty of Ljubljana, University ljubljana, ljubljana, Slovenia ;
[3]University Medical Centre Ljubljana, Department of Genetics- University Medical Centre Ljubljana- Ljubljana- Slovenia, Ljubljana, Slovenia ;
[4]University Medical Centre Ljubljana, Department of Genetics University Medical Centre Ljubljana- Ljubljana- Slovenia, Ljubljana, Slovenia

Study question: To determine the coexisting influence of obesity and PCOS on the endometrial transcriptome in ovulatory obese PCOS patients during the window of implantation in spontaneous cycles.

Summary answer: Enrichment analyses revealed significant disruptions of inflammation, insulin signaling, fatty acid metabolism and lipotoxicity pathways in ovulatory obese PCOS endometrium compared to controls.

What is known already: Obese and PCOS women have a reduced rate of spontaneous conception even when their cycles are ovulatory indicating other factors like the uterine environment. The mechanism of this intricate interplay in which obesity modifies already different PCOS endometrium is not fully clarified. There are only a few analyses of the endometrial transcriptome in which the impact of obesity and/or PCOS on the endometrium was elucidated. The most relevant was the study of Belver et al. in which obese women showed a different endometrial gene expression compared to controls with pronounced changes of transcriptome in a combination of obesity and PCOS.

Study design, size, duration: Prospective transcriptomic analysis of endometrium using the RNA-seq method was done in university-affiliated infertility hospital between February 2017 and February 2018. Clinical information and endometrial samples were collected from 14 infertile patients that were divided in 2 groups: ovulatory obese PCOS (ovul-O-PCOS) and control group. The inclusion criteria for the ovul-O-PCOS group were obesity, PCOS, and infertility and for the control group were normal body mass and tubal or unexplained infertility.

Participants/materials, setting, methods: 14 patients were invoved in the study according to the study criteria. Ultrasound detection of follicular ovulation/anovulation with determination of progesterone levels on the day of endometrial biopsy. Endometrial biopsy collected between 21 and 23 of menstrual cycle. RNA isolation (RNeasy Kit, Qiagen, Germany), sequencing, library preparation and subsequent RNAseq data analyses were done.

Main results and the role of chance: According to functional and upstream analyses the most important biological processes in ovulatory obese PCOS compared to control group were related to inflammation (TNFR1 Signalling Pathway as the most important), insulin signaling and energy consumption (Insulin Receptor signaling and PI3/AKT pathway,) fatty acid metabolism (Stearate Biosynthesis I Pathway and Palmitate Biosynthesis I Pathway) and lipidotoxity.

Upstream analysis perfectly captured the pathophysiological mechanisms found through functional analysis of ovul-O-PCOS endometrium. Most of the upstream regulators were involved in some of the already mentioned mechanisms: inflammation (CASP1, EGR1, TGFb, ZAP 70), glucose metabolism and energy homeostasis regulation (insulin, insulin receptor, GSK3, MYC, IGF1), adipokines & lipid regulators (ADIPOQ, clofibrat as a lipid-lowering agent, PPARD, PPARGC1B). Additionally, the accentuated effect of estrogen (estrogen) and impaired progesterone effect (tretinoin and altretinoin- progesterone antagonists) was also found.

Limitations, reasons for caution: Since the comorbidity of obesity and PCOS was investigated, we found the duality of the pathology as the strong point of the study. The second strong point was strictly defined study population. The most important limitation could be the sample size.

Wider implications of the findings: Obesity adds the additional burden of lipotoxity of free fatty acids in endometrium to already known factors of inflammation and insulin resistance in the O-PCOS comorbid state. The only modifiable factor is weight loss that could represent a potential mechanism for normalisation of endometrium towards controls.

Trial registration number: KME 0120-491-2017

O-133 Three-dimensional ultrasonography versus diagnostic hysteroscopy in the differential diagnosis of intra-uterine benign disease. A single institution experience.

A.M.L. Gambaro[1], M. Monti[2], M.G. Piccioni[2], A. Musella[2], V. Colagiovanni[2], E. Marcoccia[2], M.C. Schiavi[2], C. Fazi[2], L. Muzii[2], L. Manganaro[3]

[1]Guy's and St Thomas' NHS Foundation Trust, Assisted Conception Unit, London, United Kingdom ;
[2]Sapienza University of Roma, Department of Obstetrical-Gynecological Sciences and Urological Sciences-, Roma, Italy ;
[3]Sapienza University of Roma, Department of Radiological Sciences, Roma, Italy

Study question: Comparison of diagnostic accuracy of three-dimensional transvaginal ultrasonography (3D-TVUS) versus hysteroscopy (HYS) in the assessment of uterine malformations and submucosal fibroids.

Summary answer: 3D-TVUS has a high detection rate for the studied pathologies, is less invasive and more cost-effective diagnostic tool.

What is known already: Both HYS and 3D-TVUS are currently used for diagnosis of uterine malformations and submucosal fibroids. Their accuracy and the indications are operator-dependent and largely variable, between 25% and 99% depending on the circumstances and the instruments used.

Study design, size, duration: Prospective observational study. From April 2010 to December 2018, 672 patients were enrolled as they met the inclusion criteria for the study.

Participants/materials, setting, methods: Patients found to have submucosal uterine fibroids or uterine congenital anomalies on a routine pelvic transvaginal ultrasound were prospectively studied. All patients underwent 3D-TVUS, HYS and pelvic MRI, each one performed by an expert operator. Women with endometrial polyps or suspected endometrial or uterine cancer were excluded. Median age was 32 years (range 23-66 years).Specificity, sensitivity, accuracy and k-index of 3D-TVUS and HYS were recorded and compared to MRI.

Main results and the role of chance: Submucosal fibroids were confirmed by MRI in 441 out of 672 (65.6%) women; uterine malformations were reported in 231 (34.4%) women. Both 3D-TVUS and HYS showed great sensitivity in uterine malformations and submucosal fibroids detection, however 3D-TVUS showed the highest specificity in detection of uterine malformations, while HYS showed the highest specificity in detecting submucosal fibroids. Moreover, 3D-TVUS had a slightly higher accuracy in the diagnosis of uterine malformations compared with HYS (96.8% vs 93.7% respectively), while k index was excellent for both diagnostic tools. The main limitation of HYS was in differential diagnosis of fundal submucosal fibroids and uterine septum/arcuate uterus.

Limitations, reasons for caution: All procedure were performed and reviewed by expert operators with state of the art diagnostic tools, so the results reported in the study might not reflect detection rates by average operators in everyday practice.

Wider implications of the findings: 3D-TVUS is a valid tool in primary detection of uterine malformations and submucosal fibroids. It could replace diagnostic HYS and/or laparoscopy in diagnosis of uterine malformations, both where intervention is not required or to guide the surgeon in preparation for the surgery.

Trial registration number: n/a

O-134 Integration of clinical factors and biomarkers for non-invasive diagnosis of endometriosis

A. Vanhie[1], D. O[2], C. Tomassetti[1], C. Meuleman[1], P. Mestdagh[3], T. D'Hooghe[2]

[1]University Hospital Leuven, Leuven University Fertility Center, Leuven, Belgium ;
[2]KU Leuven, Development and Regeneration, Leuven, Belgium ;
[3]UGent, Center for Medical Genetics, Gent, Belgium

Study question: Can integration of clinical data and ultrasound findings with biomarkers result in accurate combined clinical-biochemical diagnostic models for endometriosis?

Summary answer: In this proof-of-concept study we show that combining clinical factors and biomarkers results in a diagnostic model for that outperforms the individual factors or biomarkers.

What is known already: Diagnosis of endometriosis solely based on presence of symptoms has a low specificity and sensitivity. Transvaginal ultrasound can diagnose endometrioma's and deep endometriotic nodules, but does not rule out peritoneal endometriosis. Cancer antigen 125 (CA-125) levels have been shown to be elevated in some women with endometriosis. However, the sensitivity and specificity of CA-125 for endometriosis is low and serum CA-125 levels alone have no value as a diagnostic tool. Recently, circulating miRNAs have been described to be differentially expressed in patients with endometriosis, suggesting that they could be used for the non-invasive diagnosis of endometriosis.

Study design, size, duration: Retrospective case-control study in a cohort of 222 patients (151 women with endometriosis and 71 controls)

Participants/materials, setting, methods: Clinical and ultrasound data were extracted from the electronic patient record. Plasma was available in the biobank of the Leuven University Fertility Center. CA-125 was measured on a Roche Modular E170 instrument using a commercial assay (Roche Diagnostics,

Germany). RNA was extracted with the miRNeasy Plasma Kit and miRNA expression analysis was done by RT-qPCR using Qiagen miScript assays. Models were build using multivariate logistic regression and validated by a 10-fold cross validation.

Main results and the role of chance: We developed a diagnostic model which finally consisted of 2 clinical factors (dysmenorrhea and dyschezia), 4 biomarkers (hsa-miR-20a-5p, hsa-miR-21-5p, hsa-miR-28-5p and CA-125) and 1 interaction factor (hsa-miR-20a-5p*dyschezia). The diagnostic model had an AUC of 79% with a high sensitivity (82%) and moderate specificity (65%) at optimal cut-off (determined by the Youden-index). In the 10-fold cross validation diagnostic performance of the model dropped to an AUC of 66%.

Limitations, reasons for caution: The 10-fold cross-validation shows that an important drop in diagnostic performance could be expected when applying this model in an independent cohort.

Wider implications of the findings: Our data indicate that combined clinical-biochemical diagnostic models perform better than the individual factors. This implies that the classic design of endometriosis biomarkers studies should be reconsidered towards an approach where the bar for each individual biomarker is lowered while keeping the overall requirement of high sensitivity and specificity.

Trial registration number: not applicable

O-135 Local steroid metabolism in eutopic endometrium and corresponding endometriotic lesions: intra-patient variability

S. Xanthoulea[1], B. Delvoux[1], P. Koskimies[2], M.R. Häkkinen[3], J. Koskivuori[3], S. Auriola[3], A. Vanhie[4], C. Tomassetti[4], A. Romano[1]

[1]Maastricht University Medical Center, Gynaecology, Maastricht, The Netherlands ; [2]Forendo Pharma, Ltd., Turku, Finland ; [3]University of Eastern Finland, School of Pharmacy, Kuopio, Finland ; [4]KU Leuven, Obstetrics and gynaecology, Leuven, Belgium

Study question: Is the local steroid metabolism (including estrogens, progestogens, androgens and corticosteroids) different between the normal endometrium of patients and the endometriotic tissue from different anatomical locations?

Summary answer: Steroid levels differ between normal and endometriotic tissue. Irrespective of the location, endometriosis shows active synthesis of estrogens and sustained corticosteroid levels.

What is known already: Endometrial tissue produces locally steroids, relevant in endometriosis: estrogens control lesion establishment, progestogens oppose estrogen action, androgens are estrogen precursors and corticosteroids suppress inflammation. These events are related to endometriosis symptoms such as infertility and pain and offer novel therapeutic targets aimed at blocking local estrogen generation, such as 17beta-hydroxysteroid dehydrogenase 1 (HSD17B1).

To elucidate to what extent steroid metabolism is implicated in endometriosis, and what inter- and intra-patient variability exists, we profiled major steroids in blood and tissue (normal endometrium and endometriosis) of patients; we also determined the expression levels of major enzymes involved in local steroid metabolism.

Study design, size, duration: This is a retrospective study using biobanked frozen patient material. Eutopic endometrium, multiple endometriotic lesions from each patient, and peripheral blood of 14 women (seven in the secretory and seven in the proliferative phase) with histologically confirmed endometriosis were analysed. Endometriotic lesions originated from the uterosacral ligament/Pouch of Douglas, bladder, ovarian fossa, rectum/rectosigmoid. Patients had stage I (n=1), II (n=9), III or (n=3), or stage IV (n=1) endometriosis (ASRM classification).

Participants/materials, setting, methods: Plasma, eutopic endometrium (n=14) and endometriotic lesions (n=39) were obtained and stored following the EPHect/WERF guidelines (https://endometriosisfoundation.org/ephect/). Patients with endometriosis were not under hormonal medication for six months prior to the biopsy. RNA expression was determined by whole RNA-sequencing. Levels of major steroids were measured by Liquid Chromatography-Mass Spectrometry (LC-MS). HSD17B1 activity was measured in tissue lysates (cell-free extracts) by High Performance Liquid Chromatography (HPLC).

Main results and the role of chance: Estrogens (estrone, estradiol) were non-statistically significantly higher in eutopic and endometriotic tissues compared with blood (estradiol: 1.0 pmol/g eutopic; 3.2 pmol/g endometriotic; 0.4 pmol/mL blood – estrone: 0.3 pmol/g eutopic; 1.1 pmol/g endometriotic; 0.3 pmol/mL blood). Of note, estradiol:estrone ratios, approximately one in blood, were around three in tissue, indicating active local synthesis. 17-hydroxy-progestogens and androstenedione were over 4-fold higher in endometriotic lesions than eutopic tissue ($p<0.05$). The activity of HSD17B1 was comparable between eutopic and endometriotic tissues.

Regarding corticosteroids, active cortisol was 4-fold higher in endometriosis than in the eutopic tissue ($p<0.001$), whereas inactive cortisone was 2.5-fold lower in endometriosis ($p<0.001$). HSD11B1 (activation to cortisol) and HSD11B2 (deactivation to cortisone) mRNA levels were in line with the corticosteroid levels: HSD11B1 mRNA was higher in endometriosis, and the opposite was observed for HSD11B2 compared with the eutopic endometrium ($p<0.001$ for both enzymes). The levels of compounds acting as precursors for corticosteroid synthesis (i.e., 21-hydroxyprogesterone; 11-deoxycorticosterone) were higher in endometriosis compared with the eutopic tissue ($p<0.05$), and a number of enzymes involved in the generation of active compounds from these precursors were expressed in both eutopic endometrium and endometriotic tissue.

Limitations, reasons for caution: This is a retrospective study, that included patients with all stages of disease and with manifestation of different symptoms in a pooled analysis. Sub analyses in relation to type of endometriosis and kind of symptoms of the patient were hampered by the small patient population.

Wider implications of the findings: Steroid levels differed between tissue and blood. HSD17B1 activity was measured in most cases (irrespective to patient and tissue location), hence, inhibition of HSD17B1 is a valid strategy for lowering the local estrogen production in endometriosis. Sustained generation of anti-inflammatory cortisol may relate to the chronic inflammation associated with endometriosis.

Trial registration number: NOT APPLICABLE

O-136 Exploring the delays to diagnosis of endometriosis in the United Kingdom; a triphasic mixed-methods study

B. Karavadra[1], E. Morris[1]

[1]Norfolk & Norwich University Hospital, Obstetrics & Gynaecology, Norwich, United Kingdom

Study question: What are the reasons for the delay to diagnosis of endometriosis from a patient and healthcare worker perspective?

Summary answer: Delay was attributed to patient factors (symptom recognition, seeking help), healthcare system issues (waiting times, referral pathways) and clinician experiences (normalisation, general vs specialist gynaecologist).

What is known already: The average time to diagnosis of endometriosis in the United Kingdom is 7.5 years and It is widely known that a delayed diagnosis can have a significant impact on women quality of life and activities of daily living. In addition, repeated medical consultations prior to diagnosis can have a significant burden financially on the healthcare system.

Study design, size, duration: A triphasic study design was adopted and it involved an online questionnaire (1252 participants), semi-structured interviews with 16 participants with endometriosis and separate focus groups with 15 healthcare workers (General Practitioners, nurses and gynaecologists). A mixed methods approach was used whereby the findings were analysed by content analysis and grounded theory qualitative methodology. For the online questionnaire, women throughout the UK responded and for the qualitative aspects of the study, purposive sampling was used.

Participants/materials, setting, methods: Phase one involved developing an online questionnaire exploring women's experiences of being diagnosed with endometriosis and this was distributed via the Endometriosis UK charity website. In phase two, the findings from the phase one study were used to design a qualitative study exploring the finer details of delays in diagnosis and analysed by grounded theory. Phase three involved focus groups with healthcare workers to explore their opinions on the findings from the phase two study.

Main results and the role of chance: In phase one, 1252 women replied to the online questionnaire with an average delay to diagnosis of 5.5 years. Delay was attributed to patient factors (symptom recognition, seeking help), healthcare system issues (waiting times, referral pathways) and healthcare worker experiences (normalisation, general vs specialist gynaecologist). In phase two, 16 women took part in individual interviews. They provided significant detail into delays by highlighting the triadic combination of coping mechanisms, health seeking behaviours and the process of adjustment to symptoms. Phase three findings from 15 healthcare workers provided insight into the preconception's healthcare

workers had 'about the endometriosis patient' and the challenges of recognising abnormal symptoms.

Mixed-method's enabled a positivist and constructivist stance to be taken when exploring a complex problem like delay. The quantitative aspect enabled insight into the general factors attributed to delay, and the subsequent qualitative phase enabled in-depth exploration of these factors and the complex interaction between them.

Limitations, reasons for caution: The findings are based on participants in the United Kingdom (UK) and the respective healthcare system; therefore, the reasons for the delay to diagnosis may vary outside the UK.

Wider implications of the findings: This study provides new in-depth insight to the health-seeking behaviours, coping mechanisms and complex interaction of factors contributing to delayed diagnosis. It is hoped that the findings from this study will be used to encourage collaboration between professional bodies and patient groups to design a pre-diagnostic tool to facilitate diagnosis.

Trial registration number: Not applicable

O-137 Elevated levels of monocyte chemotactic protein-1 (MCP-1) in the follicular fluid reveals different populations among women with severe endometriosis.

P.E. Bouet[1], J.M. Chao de la Barca[2], H. El Hachem[3], S. Blanchard[4], P. Jeannin[4], P. Reynier[2], P. May-Panloup[5]

[1]Department of Reproductive Medicine, Angers University Hospital, Angers, France ; [2]Department of Biochemistry and Genetics, Angers University Hospital, Angers, France ; [3]Department of Reproductive Medicine, Clemenceau Medical Center, Beirut, Lebanon ; [4]Department of Immunology and Allergology, Angers University Hospital, Angers, France ; [5]Department of Reproductive Biology, Angers University Hospital, Angers, France

Study question: Is there a modification of the cytokine profile of the follicular fluid (FF) of women with endometriosis undergoing in vitro fertilization (IVF)?

Summary answer: In patients with endometriosis, we have identified a subtype with a high FF MCP-1 level, that is associated with an alteration of the oocyte microenvironment.

What is known already: Several factors implicated in the acute and chronic inflammatory reaction (hormones, cytokines, chemokines and markers of oxidative stress) are thought to be involved in the pathophysiology of endometriosis. Indeed, an alteration of the cytokine profile has been shown in the serum, the peritoneal fluid, the endometrial tissue, and in endometriomas of women with endometriosis. Different biological signatures have been assessed in order to better understand the pathophysiology behind the endometriosis-related infertility, using proteomics, metabolomics and the analysis of the cytokine profile. However, such studies have been scarce and have yielded disparate results.

Study design, size, duration: We performed a prospective observational study on the follicular fluid retrieved from 87 women undergoing IVF with or without intracytoplasmic sperm injection from January 2018 to February 2019 at the Angers University Hospital, France.

Participants/materials, setting, methods: The patients were divided into two groups: the endometriosis group and the control group. The endometriosis group comprised 43 patients with severe endometriosis. The control group included 44 patients undergoing IVF for other causes and without endometriosis. The cytokine profile of the FF was determined by multiplex fluorescent-bead-based technology allowing the measurement of 59 cytokines.

Main results and the role of chance: Univariate analysis showed a significant increase of nine cytokines in the FF in the endometriosis group compared to the control group, while Monocyte Chemoattractant Protein 1 (MCP-1) was the only variable retained in the multivariate analysis. We identified two subgroups of patients in the endometriosis group: MCP-1- group (n=23) which had FF MCP-1 levels comparable to the control group and MCP-1+ (n=20) which had significantly higher FF levels. Only patients in the MCP-1+ group had a significantly altered cytokine profile in the FF, and had a significantly higher serum estradiol level (p=0.002) and a significantly lower number of oocytes recovered (p=0.01) compared to the MCP-1- and the control group.

Limitations, reasons for caution: One potential limitation of our study is the lack of assessment of serum MCP-1 levels, which could have been compared to those in the follicular fluid.

Wider implications of the findings: Our study has shown an alteration of the oocyte microenvironment in women with endometriosis associated with high follicular fluid levels of MCP-1 allowing the identification of a subgroup of endometriosis patients with a potentially worse prognosis.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 35: IMPACT OF ART ON HEALTH OUTCOMES OF CHILDREN

07 July 2020 Parallel 5 10:00 - 11:30

O-138 Differences in the landscape of mitochondrial DNA variants between ART and spontaneously conceived individuals links to maternal infertility and low birth weight

J. Mertens[1], F. Zambelli[2], F. Belva[3], A. Van Montfoort[4], E. Couvreau de Deckersberg[1], M. Bonduelle[3], K. Stouffs[3], S. Seneca[3], H. Van de Velde[5], C. Blockeel[5], K. Barbé[6], K. Sermon[1], C. Spits[1]

[1]Vrije Universiteit Brussel, Reproduction and Genetics, Brussels, Belgium ; [2]Clinica Eugin, Clinica Eugin, Barcelona, Spain ; [3]UZ Brussel, Center for Medical Genetics, Brussels, Belgium ; [4]Maastricht University Medical Center, Department of Obstetrics & Gynaecology-GROW School for Oncology and Developmental Biology, Maastricht, The Netherlands ; [5]UZ Brussel, Center for Reproductive Medicine, Brussels, Belgium ; [6]Vrije Universiteit Brussel, Interfaculty Center Data Processing & Statistics ICDS, Brussels, Belgium

Study question: Can mitochondrial DNA variants explain the differences in birth weight between ART and spontaneously conceived individuals?

Summary answer: We have found significant differences in the mitochondrial genome of children born after ART, which link to a background of maternal infertility and birth weight.

What is known already: Follow-up studies of children born after ART show an increased risk of lower birth weight and the potential development of an abnormal cardio-metabolic profile later in life. Efforts to find the molecular cause of these differences have focused on the epigenome, but have yet to yield conclusive results. In this study, we investigated variation in the mitochondrial genome, which is known to be linked to cardio-metabolic disorders in the general population and potentially to infertility. Our hypothesis was that mitochondrial DNA variants could explain the differences in birth weight between ART and spontaneously conceived individuals.

Study design, size, duration: We studied the full mitochondrial DNA (mtDNA) of 283 ART and 189 spontaneously conceived (SC) samples collected in multiple centers collaborating with large fertility clinics. The DNA was extracted from different tissues (ART: 116 blood, 66 placental and 101 buccal samples, SC: 65 blood, 51 placental and 73 buccal samples). The DNA was enriched for the mtDNA by long-range PCR and sequenced on an Illumina platform.

Participants/materials, setting, methods: The variant calling was performed using mtDNA server and MuTect, with a threshold of >1.5% load, versus the reference NC_012920.1. The results were correlated to maternal age, indication for ART, culture medium, gestational age, and birth weight corrected for gestational age and gender. Statistical analysis was done using SPSS. For the complex data of the heteroplasmic variants, an orthogonally rotated factor analysis was used to reduce the dimensionality of the studied dependent variables.

Main results and the role of chance: Haplogroup U4 was overrepresented in the ART group (ART: 5.3% vs. SC: 0.5%, p=0.004). ART individuals have more not previously described homoplasmic variants (ART: 31.4%, SC: 21.7%, p=0.021). ART Individuals whose mother was infertile more frequently carried homoplasmic tRNA variants than SC or individuals with infertile fathers (p=0.041). ART individuals carry higher heteroplasmic loads in the coding region (p=0.039) and component analysis, using rank randomized tests, indicated that they carry a different mtDNA variant composition from SC individuals (p=0.015),

which is driven by variants inducing non-synonymous changes and in the rRNA regions. These heteroplasmic variants were highest in individuals born from infertile mothers, followed by male factor infertility when compared to the SC group (significant trend, p=0.017). Maternal age nor gestational age were linked to an increased mutational burden. Finally, both ART and SC individuals ≤10th birth weight percentile more frequently carried homoplasmic tRNA variants (p=0.032). SC individuals with birth weight ≤10th and ≤25th percentile carried more frequently heteroplasmic coding variants (p=0.032 and p=0.008). In the ART group, only individuals from embryos that had been in Vitrolife G1 medium followed the same pattern as SC, with individuals ≤25th percentile carrying more heteroplasmic coding variants (p=0.0232).

Limitations, reasons for caution: Other factors not considered in this study could play a role in the birth weight, which we are currently correcting for. This study is observational and no functional tests were performed. Finally, after stratification for embryo culture medium, the sample sizes are small (N=49- 66).

Wider implications of the findings: These results suggest a link between maternal infertility and mtDNA variant composition, which is transmitted to the offspring. These variants correlate to birth weight, particularly in the SC individuals, suggesting a universal mechanism. Embryo culture medium appears to affect birth weight through another mechanism, masking the effect of mtDNA variation.

Trial registration number: not applicable'

O-139 Imprinting disorders in children born after assisted reproductive technology (ART): a Nordic study from the CoNARTaS group

Ø. Lidegaard[1], A.K.A. Henningsen[2], M. Gissler[3], S. Rasmussen[4], S. Opdahl[5], U.B. Wennerholm[6], A.L. Spangsmose[7], A. Tiitinen[8], C. Bergh[6], L.B. Romundstad[9], H. Laivuori[10], J. Forman[11], A.B. Pinborg[7]

[1]Rigshospitalet- University of Copenhagen, Dept. of Ob/Gyn, Copenhagen, Denmark ;
[2]Rigshospitalet- University of Copenhgen, Fertility Clinic, Copenhagen, Denmark ;
[3]THL Finnish Institute for Health and Welfare-, Information Services Department, Helsinki, Finland ;
[4]Rigshospitalet- University of Copenhagen, Department of Gynaecology, Copenhagen, Denmark ;
[5]Central Norway Regional Health Authority- Norwegian University of Science and Technology-, Department of Public Health and General Practice, Oslo, Norway ;
[6]Institute of Clinical Sciences- Sahlgrenska Academy- University of Gothenburg-, Department of Obstetrics and Gynecology, Gothenburg, Sweden ;
[7]Rigshospitalet- Copenhagen University Hospital-, Fertility Clinic, Copenhagen, Denmark ;
[8]University of Helsiniki- Helsinki University Hospital -, Department of Obstetrics and Gynecology, Helsinki, Finland ;
[9]Central Norway Regional Health Authority- Norwegian University of Science and Technology-, Department of Public Health and General Practice- Spiren Fertility Clinic, Oslo, Norway ;
[10]University of Helsinki- Institute for Molecular Medicine- Institute of Life Science- University of Helsinki- Helsinki University Hospital- Medical and Clinical Genetics- Faculty of Medicine and Health Technology- University of Tampere- Tampere Univers, , ;
[11]University of Copenhagen-, Department of Biostatistics, Copenhagen, Denmark

Study question: Is the risk of imprinting disorders increased in children conceived after assisted reproductive technology (ART)?

Summary answer: We found an odds ratio of 3.07 [95%CI 1.49-6.31] for Beckwith-Wiedemann syndrome in ART children, but no increased risk of three other imprinting disorders.

What is known already: Earlier studies, most of them small, have suggested an association between ART and imprinting disorders. Results, however, far from consistent.

Study design, size, duration: Binational register-based cohort study. All children conceived by ART in Denmark (n=45 393) born between 1994 and 2014 and in Finland (n=29 244) born between 1990 and 2014 were identified. The full background populations born during the same time periods in the two countries were included as controls. Odds ratios of imprinting disorders in ART children compared with naturally conceived (NC) children were calculated.

Participants/materials, setting, methods: From the national health registries in Denmark and Finland, we identified all children diagnosed with Prader-Willi syndrome (n=143); Silver-Russell17 syndrome (n=69); Beckwith-Wiedemann syndrome (n=105) and Angelman syndrome (n=72) born between 1990/1994 and 2014, respectively. he median follow-up time was 8 years and 9 months for ART children and 11 years and 9 months for NC children.

Main results and the role of chance: We identified a total of 389 children diagnosed with imprinting disorders, sixteen of these were conceived after ART. The overall odds ratio for the four imprinting disorders in ART children compared with NC children was 1.60 [95%CI 0.97-2.65]. Made up separately, eight ART children were diagnosed with Beckwith-Wiedemann syndrome, the odds ratio for this specific imprinting disorder was 3.07 [95%CI 1.49-6.31]. The risk of Prader-Willi syndrome, Silver-Russell syndrome and Angelman syndrome was not increased in children conceived after ART.

Limitations, reasons for caution: Imprinting disorders are rare events and our results based on few ART children with imprinting disorders. The etiology is complex and only partly clarified, and the clinical diagnoses challenged by a broad phenotypic spectrum.

Wider implications of the findings: In the existing studies results on the risk of imprinting disorders in children conceived after ART are ambiguous. This study adds that the risk of imprinting disorders in ART children is very small and perhaps restricted to Beckwith-Wiedemann syndrome

Trial registration number: not applicable

O-140 ICSI does not increase the odds of adverse perinatal outcomes in autologous/ donor oocyte cycles without male-factor subfertility: analysis of 121,448 singleton live births

P.R. Supramaniam[1], L.N. Lim[1], E. McVeigh[1], I. Granne[1], C. Becker[1], M. Mittal[2]

[1]Oxford University Hospitals NHS Foundation Trust, Gynaecology, Oxford, United Kingdom ;
[2]Imperial College Healthcare, Gynaecology, London, United Kingdom

Study question: Does ICSI increase the odds of adverse perinatal outcomes in autologous and donor oocyte cycles without male factor subfertility?

Summary answer: ICSI does not increase the odds of adverse perinatal outcomes in autologous and donor oocyte cycles in the absence of male factor subfertility.

What is known already: An increased incidence of obstetric complications is recorded in pregnancies conceived through assisted reproductive treatments (ART) compared to spontaneous conception. There is a paucity of information is available on the effect of method of fertilisation, particularly ICSI, on perinatal outcomes in the absence of male factor subfertility in either autologous or donor oocyte cycles.

Study design, size, duration: Retrospective cohort analysis of all fresh cycles conducted between 2002-2016, from the UK HFEA national database. 110,805 singleton live births(LB) have been recorded from all oocyte usage, of which, 105,618 were from autologous oocyte cycles and 5187 from donor oocyte cycles. Assuming that the adverse perinatal outcome rate for IVF is 10%, a power calculation demonstrated that 686 LBs would need to be analysed for 80% power and 5% significance to detect a 10% difference.

Participants/materials, setting, methods: The database was analysed for singleton live birth rate (SLBR), stratified by recipient age, donor age, number of previous IVF treatment cycles undertaken and type of cycle (fresh IVF versus fresh ICSI). Cycles complicated by male factor subfertility were excluded from the final analysis as this is known to influence the method of fertilisation. Statistical analysis was performed using Logistic Regression and Chi-square; p<0.05 was considered statistically significant.

Main results and the role of chance: The overall IVF to ICSI ratio for all treatment cycles, in the absence of male factor subfertility, favoured IVF (58:42) in 2002, with a yearly rise seen in ICSI cycles (46:54 in 2016).

IVF increased the risk of very preterm births (<32 weeks') (adjusted odds ratio [aOR] 1.25, 99.5% confidence interval [CI] 1.10-1.42, p<0.0001) and preterm births (32-36 weeks') (aOR 1.10, 99.5% CI 1.03-1.17, p<0.0001) compared to ICSI treatment cycles with autologous oocytes, using term delivery as the reference category. This significance was not sustained in donor oocyte cycles: very preterm births (aOR 1.17, 99.5% CI 0.70-1.96, p=0.393) and preterm births (aOR 0.90, 99.5% CI 0.69-1.17, p=0.250). The odds of a

post-dates delivery was increased in autologous oocyte cycles employing ICSI when compared to IVF (aOR 1.05, 99.5% CI 1.01-1.10, p=0.003), however, this was not maintained in donor oocytes (aOR 0.94, 99.5% CI 0.75-1.19, p=0.459). There was no significant difference demonstrated when the birthweight per method of fertilisation was analysed after adjusting for potential confounding factors in either oocyte category

Limitations, reasons for caution: The accuracy of the database is dependent on the information submitted to the HFEA. Until 2007, this data was manually captured adding the risk of data entry error. Furthermore, information on maternal confounding factors such as body mass index and smoking status was not available to be evaluated.

Wider implications of the findings: ICSI in the absence of male factor subfertility does no increase the odds of adverse perinatal outcome in autologous and donor oocyte cycles. This at present suggests that the adverse outcomes previously described with ICSI is related to the significant abnormalities associated with the male component rather than the technique.

Trial registration number: N/A

O-141 The risk of major congenital malformations in children conceived after ICSI: a Nordic cohort study

A.K. Henningsen[1], S. Opdahl[2], U.B. Wennerholm[3], A. Tiitinen[4], L.B. Romundstad[5], C. Bergh[3], M. Gissler[6,7], J. Forman[8], A. Pinborg[1]

[1]Rigshospitalet, Fertility Clinic, Copenhagen, Denmark ;
[2]Norwegian University of Science and Technology, Department of Public Health and Nursing, Trondheim, Norway ;
[3]Institute of Clinical Sciences- Sahlgrenska Academy- University of Gothenburg-Sahlgrenska University Hospital, Department of Obstetrics and Gynecology, Gothenburg, Sweden ;
[4]University of Helsinki- Helsinki University Hospital-, Department of Obstetrics and Gynecology, Helsinki, Finland ;
[5]Spiren Fertility Clinic, Spiren Fertility Clinic, Trondheim, Norway ;
[6]THL Finnish Institute for Health and Welfare, Information Services Department, Helsinki, Finland ;
[7]Karolinska Institute- Division of Family Medicine, Department of Neurobiology-Care Sciences and Society, Stockholm, Sweden ;
[8]University of Copenhagen, Department of Biostatistics, Copenhagen, Denmark

Study question: Is the risk of major congenital malformations higher in children conceived after ICSI compared with children conceived after IVF?

Summary answer: We did not find any difference in the risk of being born with a major congenital malformation in children conceived after ICSI compared with IVF.

What is known already: It is well known that children born after ART have a higher risk of being born with a major congenital malformation compared with children born after natural conception (NC). It is still not clear whether the risk of congenital malformations is higher in children conceived after ICSI compared with IVF, as studies so far present diverging findings. It is also uncertain if cryopreservation of the embryos influences the risk of malformations in the offspring.

Study design, size, duration: Nordic cohort study based on national registry data from Denmark, Norway and Sweden. The analyses were based on singletons born after ART (n=98 026) and NC (n=4 804 844) between 1992 and 2015.

Participants/materials, setting, methods: The study included all singletons, both live-born and stillborn, conceived after ICSI (n=39 684); IVF (n=58 342) and NC (n=4 804 844). We differentiated between children conceived after transfer of fresh embryos versus frozen-thawed embryos. Malformations were coded in all countries using the International Classification of Diseases (ICD). The European Concerted Action on Congenital Anomalies and Twins (EUROCAT) was used to differentiate between major and minor malformations. Minor malformations were not included in the analyses.

Main results and the role of chance: I the multiple regression analyses, we adjusted for maternal age, parity, year of birth, child's sex, body mass index, smoking and country. We found no difference in the risk of being born with a major congenital malformation, when comparing children conceived after ICSI with children conceived after IVF, adjusted odds ratio (AOR) 1.06; [95% confidence interval (CI) 0.98-1.13]; p-value=0.09. Neither could we detect any difference in the risk of major malformations when comparing children born after transfer of a fresh ICSI embryo versus a frozen-thawed ICSI embryo, AOR 1.11; [95%CI, 0.98-1.26]; p-value=0.09. We also compared children conceived after transfer of frozen-thawed ICSI embryo with a frozen-thawed IVF embryo. The risk of major malformations was similar for the two groups, AOR 1.04; [0.90-1.21]; p-value=0.57. When we investigated the risk of malformations grouped by different organ systems, we did not find higher risk among ICSI children in any of the above comparisons. However, we confirmed that children born after ICSI have a significantly higher risk of being born with a major malformation compared with NC children, AOR 1.30; [95%CI 1.24-1.36]; p-value<0.0001.

Limitations, reasons for caution: Due to the poorer quality of registration of minor malformations in the national health registries, together with surveillance bias, we restricted our analyses to major malformations. We do not expect the detection of major congenital malformation to be biased by method of conception.

Wider implications of the findings: Since the first ICSI child was born in 1992, there has been concern whether the risk of congenital malformations is increased among ICSI children, where often poor-quality semen has been used for conception. Our findings based on 39 684 Nordic ICSI children, showing no differences compared to IVF, are reassuring.

Trial registration number: 71450

O-142 The DNA methylation patterns within whole blood of adolescents born from IVF are not different from those adolescents conceived naturally within the Raine birth cohort.

B. Penova-Veselinovic[1], P. Melton[2], R.C. Huang[3], J. Yovich[4], P. Burton[5], R. Hart[6]

[1]University of Western Australia, Division of Obstetrics and Gynaecology, Perth, Australia ;
[2]University of Western Australia, Centre for Genetic Origins of Health and Disease- Statistical Genetics, Perth, Australia ;
[3]University of Western Australia, Telethon Kids Institute, Perth, Australia ;
[4]Curtin University, PIVET Medical Centre, Perth, Australia ;
[5]Edith Cowan University, Concept Fertility Centre, Perth, Australia ;
[6]University of Western Australia & Fertility Specialists of WA, School of Women's and Infants Health, Perth- Western Australia, Australia

Study question: Do the epigenome-wide DNA methylation profiles of adolescents born from in-vitro fertilisation (IVF) differ from the epigenome of naturally conceived counterparts from a birth cohort?

Summary answer: There is no significant difference in the DNA methylation profiles of adolescents born from IVF when compared to their naturally conceived, age-matched counterparts.

What is known already: Epigenetic changes are heritable modifications that may alter gene expression without changing the DNA sequence. Growing evidence suggests that the adverse health outcomes reported in IVF-born offspring might have underlying epigenetic mechanisms. Both, features of infertile couples, as well as the IVF procedure itself have been shown to alter the epigenetic signature in offspring and placental tissue. As most studies investigated DNA methylation changes in cord blood/placenta tissue, and a recent study reported that these changes are mitigated by adulthood, it is essential to further investigate potential effects of IVF on the DNA methylation profiles in adolescents using whole blood.

Study design, size, duration: The Growing Up Healthy Study (GUHS) is a prospective study that recruited 303 adolescents to compare various long-term health outcomes and DNA methylation profiles with age-matched counterparts from a birth cohort (Generation 2 of the Raine Study). GUHS assessments were conducted in 2013-2017. The effect of IVF on DNA methylation levels of 238 adolescents mean age 16±1.67 (52.94% male) was compared to 1188 naturally conceived, age-matched controls, 17.25±0.58 (50.93% male) from the Raine Study.

Participants/materials, setting, methods: Genomic DNA from whole blood was used to generate the epigenome profiles of 238 GUHS adolescents and 14 technical replicates using the Infinium Methylation Epic Bead Chip that measures and quantifies approximately 850,000 DNA methylation probes. These generated profiles were compared to the DNA methylation profiles from the Raine Study that was quantified using Illumina 450K platform. DNA methylation profiles for both studies were normalized using the same quantile normalization procedure (BMIQ) for comparative purposes.

Main results and the role of chance: We defined adolescence by an age range between 13 and 19.9 years. The average age of the generated DNA

methylation profiles in the GUHS cohort was 16.06 years. The DNA-methylation profiles of the Raine Study participants have an average age of 17.25 years. After quality control and filtering, a total of 401,022 DNA methylation probes overlapped between the two studies. These DNA methylation probes were then each investigated and compared between the IVF born GUHS and naturally conceived Raine study adolescents. We tested for an association between these groups applying Firth's bias reduced logistic regression against the outcome of IVF vs naturally conceived. Potential confounders, namely the DNA methylation probe as well as technical variation due to different methylation platforms used between studies, were adjusted for within-study batch effects. After adjustment none of the compared DNA methylation probes reached a Bonferroni correction of 1.24E-0.7 (0.05/402,022) for statistical significance. Overall, a small minority, 3850 (0.96%) of the analyzed 401,022 DNA methylation probes showed nominal significance with a p-value <0.05, most likely to be false positives after controlling for cross-study comparisons. 1,810 Differentially Methylated Regions (DMR's) were identified between the cohorts; however none reached statistical significance after correcting for multiple testing.

Limitations, reasons for caution: With contradictory findings on small sample size studies to date, there is a need to better understand the complex outcomes and effects of IVF manipulations on the epigenome of offspring. To infer an association with higher statistical and biological significance, data from various groups should be pooled for a meta-analysis.

Wider implications of the findings: Our results support previous findings that the epigenome profiles of IVF born adolescents are stable, and not affected by the techniques used in IVF. The increased cardiometabolic risk factors observed in adolescents born from IVF treatment may result from other non-DNA methylation related epigenetic influences.

Trial registration number: NHMRC Project grant number 1042269

O-143 Female causes of infertility are associated with higher risk of preterm birth and low birth weight: analysis of 120 902 singleton live births following IVF

M.S. Kamath[1], B. Antonisamy[2], S.K. Sunkara[3]

[1]Christian Medical College and Hospital, Reproductive Medicine, Vellore, India ; [2]Christian Medical College and Hospital, Biostatistics, Vellore, India ; [3]King's College London-, Division of Women's Health- Faculty of Life Sciences and Medicine-, London, United Kingdom

Study question: Does cause of infertility affect perinatal outcomes of preterm birth (PTB) and low birth weight (LBW) following In vitro fertilisation (IVF) treatment?

Summary answer: The risk of PTB and LBW was significantly higher with female causes of infertility – ovulatory disorders, tubal disorders and endometriosis compared to unexplained infertility.

What is known already: Women with infertility have an increased risk of adverse perinatal outcomes. Risk of adverse perinatal outcomes is also higher following assisted reproductive treatments (ART) compared to spontaneous conceptions. Infertility can result from female and or male factors or unexplained when the cause cannot be delineated by standard investigations. Given that infertility and ART are contributory to the adverse perinatal outcomes, it is a matter of interest to delineate if the specific cause of infertility influences the perinatal outcomes following IVF treatment.

Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA). The HFEA has collected data prospectively on all ART cycles performed in the UK since 1991. Data from 1991 to 2016 comprising a total of 120 902 singleton live births following IVF ± ICSI for sole causes of infertility were analysed for PTB and LBW. Cycles having more than one cause of infertility and multiple births were excluded.

Participants/materials, setting, methods: Data on all women undergoing stimulated IVF ± ICSI treatment cycle were analysed to compare perinatal outcomes of PTB and LBW among singleton live births based on the cause of infertility (ovulatory disorders, tubal disorders, endometriosis, male factor, unexplained). Logistic regression analysis was performed adjusting for female age category, period of treatment, previous live births, IVF or ICSI, number of embryos transferred and fresh or frozen embryo transfer cycles.

Main results and the role of chance: The incidence of PTB following IVF based on infertility causes was 10.8% for ovulatory disorders; 10.5% for tubal disorders; 9.8% for endometriosis; 8.1% for male factor causes and 8.5% for unexplained infertility. Compared to unexplained infertility cause, the risk of PTB was significantly higher with ovulatory disorders (adjusted odds ratio-aOR 1.31, 95% CI 1.21, 1.41); tubal disorders (aOR 1.25, 95% CI 1.18, 1.34) and endometriosis (aOR 1.17, 95% CI 1.05, 1.29). There was no significant difference in the risk of PTB with male factor causes compared to unexplained infertility (aOR 1.25, 95% CI 1.18, 1.34).

The incidence of LBW based on infertility causes was 10.5% for ovulatory disorders; 9.4% for tubal disorders; 9.8% for endometriosis; 8.4% for male factor causes and 8.5% for unexplained infertility. Compared to unexplained infertility cause, the risk of LBW was significantly higher with ovulatory disorders (aOR 1.29, 95% CI 1.20, 1.40); tubal disorders (aOR 1.12, 95% CI 1.05, 1.20) and endometriosis (aOR 1.11, 95% CI 1.0, 1.24). The risk of LBW was significantly lower with male factor causes compared to unexplained infertility (aOR 0.94, 95% CI 0.89, 0.99).

Limitations, reasons for caution: Although the analysis was adjusted for several important confounders, there was no information on medical history of women during pregnancy to allow adjustment. Limitations with observational data would apply to this study including residual confounding.

Wider implications of the findings: This is the first study to address causes of infertility affecting perinatal outcomes of PTB and LBW. The information is important and needs to be understood further.

Trial registration number: Not applicable

O-144 The risk of cerebral palsy in ART children has more than halved over two decades – a Nordic collaborative study on 55,233 liveborn children

A.L. Spangmose[1], L. Hee[1], M. Gissler[2], A. Tiitinen[3], S. Opdahl[4], L.B. Romundstad[5], C. Bergh[6], U.B. Wennerholm[6], A.K. Henningsen[1], A. Pinborg[1]

[1]Fertility Clinic Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark ; [2]Information Services Department- Finnish Institute for Health and Welfare THL, Department of Neurobiology- Care Sciences and Society- Karolinska Institute, Helsinki and Stockholm, Finland ; [3]Helsinki University Hospital and University of Helsinki, Department of Obstetrics and Gynecology, Helsinki, Finland ; [4]Norwegian University of Science and Technology, Department of Public Health and Nursing, Trondheim, Norway ; [5]Norwegian Institute of Public Health, Spiren Fertility Clinic- Center for Fertility and Health, Trondheim, Norway ; [6]Institute of Clinical Science- Sahlgrenska Academy, Department of Obstetrics and Gynecology, Gothenburg, Sweden

Study question: Has the risk of cerebral palsy (CP) changed over a 20-year period in children born after assisted reproductive technology (ART)?

Summary answer: Over two decades, the risk of CP in ART-children decreased with more than 50%, mainly due to single embryo transfer resulting in lowering twin rates.

What is known already: During the last two decades we have seen a considerable reduction in twin birth rates after ART treatment in Europe herein the Nordic countries. In the Nordic countries, the twin births rates in ART pregnancies have declined from almost 25% two decades ago to less than 5%, compared with 2–3% in the background population of spontaneously conceived (SC) pregnancies. Concomitantly the preterm birth (PTB) rate has diminished considerably. PTB increases the risk of CP, which is one of the most severe complications in ART-children with long term consequences for the children and their families.

Study design, size, duration: A Nordic registry-based cohort study using data from Denmark (birth years 1994–2010) and Finland (1990–2010) including two cohorts: 55,233 ART-children and 2,327,350 SC-children. Among the ART-children, 37,404 were singletons, 17,057 were twins and 772 were higher order multiples. We investigated the risk of CP in the following time periods: birth year 1990–1994, 1995–1999, 2000–2004 and 2005–2010. Children were followed up until 2014.

Participants/materials, setting, methods: Data originated from the CoNARTaS cohort (Committee of Nordic ART and Safety) containing

information from national ART- and patient registries. CP was defined as G80 ICD-10-diagnoses registered in the patient registry before the age of ten. Risk of CP was compared for ART vs. SC children, singletons and twins using logistic regression models. Adjustments were made for maternal age, parity, child's sex, country and birth year as well as plurality (if applicable).
Main results and the role of chance: Overall, 307 (0.6%) ART- and 5,911 (0.3%) SC-children were diagnosed with CP. During the study period the crude risk of CP in ART-children decreased consistently from 0.9% (1990–1994) to 0.4% (2005–2010), while the risk remained unchanged for SC-children (0.3%). For ART-singletons the risk of CP decreased from 0.7% (1990–1994) to 0.3% (2005–2010), but remained stable for ART-twins (0.7%), SC-singletons (0.2%) and SC-twins (0.8%). Throughout the study period the adjusted risk of CP was higher for ART- versus SC-children (adjusted odds ratio [aOR] 1.93 [95%CI 1.71;2.17]). The risk remained increased after further adjustment for plurality (aOR 1.18 [95%CI 1.04;1.34]). The risk of CP was higher for ART-singletons (aOR 1.32 [95% CI 1.10;1.57]) but similar for ART-twins compared with their SC counterpart. Analyses stratified on birth year showed a consistent decrease in risk of CP over time for ART- versus SC-children (1990–1994: aOR 2.88 [95%CI 1.81;4.32]; 2005–2010: aOR 1.34 [95%CI 1.12;1.61]). In 2005–2010 the risk of CP, after further adjustments for plurality, was no longer statistically significant (aOR 0.96 [95%CI 0.79;1.15]). Additionally, the risk of CP decreased substantially over time for ART-singletons compared with SC-singletons (1990–1994: aOR 2.53 [95%CI 1.20;4.63]; 2005–2010: aOR 1.21 [95%CI 0.88;1.62]).
Limitations, reasons for caution: Observational studies may have inadequate adjustment for potential confounding factors. Despite adjustment for some major confounders, residual confounding cannot be excluded.
Wider implications of the findings: Limiting the number of twins born after ART treatment lowers the risk of CP in the ART population. Multiple embryo transfer is still the standard care in many countries. Our findings emphasize that single embryo transfer should be encouraged worldwide.
Trial registration number: ISRCTN11780826

INVITED SESSION
SESSION 36: COVID-19-LESSONS LEARNED
07 July 2020 Parallel 6 10:00 - 11:30

O-145 Modelling the COVID-19 epidemic and implementation of population-wide interventions in Italy

G. Giordano[1]

[1]*Universita de Trento, Dipartimento di Ingegneria Industriale, Italie*

O-146 Is COVID-19 symptomatic triage enough? The limited value of serological testing

C. González-Ravina[1], **V. Vergara**[2], **M. Cruz**[2], **N. Prados**[3], **A. Requena**[2]

[1]*IVI Sevilla, Andrology and Endocrinology laboratories, Sevilla, Spain*
[2]*IVI Madrid, Reproductive Medicine, Madrid, Spain*
[3]*IVI Sevilla, IVF Laboratory, Sevilla, Spain*

Study question: Do serological and molecular tests increase the detection of patients with SAR-CoV-2 after a negative triage?
Summary answer: Serological testing has limited value in detecting SARS-CoV-2 once triage has been negative if molecular testing is being performed before oocyte retrieval or embryo transfer
What is known already: Fertility community is uncertain about how to optimally provide care to infertile patients, without compromising safety, once the activity is re-established. The key principle established by the scientific societies for resuming activity is that anyone attending a clinic should be triaged negative. More precise methodology has been developed such as immunological tests that inform us of the state of the disease and molecular tests (RT-PCR) that measures viral load. These tests may help us to identify asymptomatic and pre-symptomatic carriers that could have not been detected only with triage and patients with past infections that may need no further tests.
Study design, size, duration: Between April 27th and May 19th, 2020, 1549 women were tested for SARS-CoV-2. Before these tests were performed, a symptomatic triage had been carried out in which patients were asked by telephone for the presence of symptoms of the virus or if they had been in contact with someone suspected or confirmed to have been infected with the disease. Only patients classified as negative triage attended the clinic for further testing.
Participants/materials, setting, methods: IgG and IgM antibody against SARS-CoV-2 in plasma samples were tested using enzyme linked immunosorbent assay (ELISA) kits (Epitope Diagnostics, USA). Women with asymptomatic triage and negative IgM started a treatment; on the 5th-8th day of stimulation for retrieval or 3-5 days before a frozen embryo transfer, a nasopharyngeal RT-PCR was performed in order to avoid an active infection during the treatment.
Main results and the role of chance: Study was performed in 17 private clinics belonging to IVIRMA group. Serological testing was carried out in 1549 patients before starting a treatment. Seroconversion rate for IgG was 3.8% (n=59) and for IgM was 0.7% (n=11). As previously discusses, only those patients in whom the recent presence of the infection had been ruled out (IgG+/IgM- or IgG-/IgM-) continued with the treatment. Before embryo transfer, both in fresh and frozen cycles, a molecular determination of the virus was carried out and we observed a 0.06% (n=1) rate of positive RT-PCR.

The possibilities of having a patient with negative IgG and IgM and a positive RT-PCR is due to the period between the pre-symptomatic or asymptomatic shedding and the first time at which the production of IgM starts, which can be up to 12 days. Even though a negative RT-PCR could be considered enough as immunity is not well established, there is no clear evidence that past infections could be re-infected, and IgG seem to be neutralizing. Serological testing of IgM prevents patients from starting endometrial priming or controlled ovarian stimulation decreasing the risk of cancelling the cycle while a positive IgG avoids repeating RT-PCR for each oocyte retrieval or embryo transfer
Limitations, reasons for caution: The low prevalence of infected patients after the triage makes these number very approximate. The utility of these tests will worsen as the virus incidence decreases.
Wider implications of the findings: In this current scenario, serological testing does not seem to be a cost-effective strategy to avoid asymptomatic carriers of the virus for starting a treatment, but it still detects a low number of asymptomatic patients. Lower occurrence of the virus may suggest the no need for a later screening.

O-147 Seroconversion of Immunoglobulins to SARS-CoV-2 of healthcare workers in nine European fertility units

S. Nelson M.B.B.Ch- Ph.D.[1], **S. Ehnert**[2], **T. Child**[3], **G. Trew**[4]

[1]*University of Glasgow, Level 2 New Lister Building, Glasgow, United Kingdom*
[2]*VivaNeo Laborgiagnostik A member of The Fertility Partnership, Laborgiagnostik, Düsseldorf, Germany*
[3]*The Fertility Partnership, Oxford Fertility, Oxford, United Kingdom*
[4]*The Fertility Partnership, Boston Place, London, United Kingdom*

Study question: To assess SARS-CoV-2 seroconversion in healthcare workers in nine European IVF units on recommencement of clinical activity.
Summary answer: A large proportion of staff remain susceptible to SARS-CoV-2 infection with no evidence of seroconversion. Comprehensive risk mitigation strategies are essential for continued staff welfare.
What is known already: The diagnosis of COVID-19 is based on the detection of the SARS-COV2 virus using RT-PCR from nasopharyngeal samples at the time of active infection. Most patients infected with SARS-COV-2 develop antibodies (Ab) against SARS-COV2 proteins. Regulatory approved CE marked commercial ELISAs are now available to assess seroconversion, yet the prevalence of SARS-CoV-2 antibodies amongst healthcare workers in IVF units is unknown.
Study design, size, duration: Prospective cohort study of 175 staff members from 8 European IVF clinics and one diagnostic laboratory (5 Germany, 3 Austria), sampled over a two-week period with paired SARS-CoV-2 antigen testing and blood sampling for serology.
Participants/materials, setting, methods: Staff returning to work in the nine European clinics of The Fertility Partnership received paired oropharyngeal antigen testing for RT-PCR and blood sampling for serology. RT-PCR for SAR-CoV-2 was performed in accordance with WHO guidelines. Detection of SARS-CoV-2 antibodies was performed by the Abbott Diagnostics SARS-CoV-2 IgG

assay on an Abbott Architect i2000 according to the manufacturer's instructions. This qualitative assay detects IgG binding to an undisclosed epitope of the SARS-CoV-2 nucleocapsid protein.

Main results and the role of chance: Of the 175 staff members tested 0 (95%CI 0.0 to 2.1%) had evidence of SARS-CoV-2 as detected by RT-PCR. In contrast 6 healthcare workers 3.4% (95%CI 1.6 to 7.3%) had antibodies against SARS-CoV-2. There was no evidence of clustering within the clinics with four of the nine facilities having only one staff member affected (prevalence estimates ranging from 5.6% to 12.5%), and one clinic having three staff members affected (9.1% (95%CI 3.1% to 23.6%).

Limitations, reasons for caution: Understanding viral and host interactions during acute and convalescent phases are critical to interpret both the timing of initial seroconversion after exposure to SARS-CoV-2, and the subsequent duration of antibodies. At present studies regarding temporal seroconversion have been developed in conjunction with assay development, limiting the long-term conclusions of seroconversion.

Wider implications of the findings: A low proportion of healthcare workers in nine different sites had seroconverted and had evidence of SARS-CoV-2 antibodies. A large proportion of staff are still susceptible to infection and appropriate infection control procedures are essential to continue to mitigate risk and ensure staff welfare with the resumption of clinical services.

O-315 Streamlining follicular monitoring during controlled ovarian stimulation? A data-driven approach to efficient IVF care in the new era of social distancing

I. Robertson[1], F.P. Chmiel[2], Y. Cheong[1]

[1]University of Southampton, Human Development and Health, Southampton, United Kingdom

[2]University of Southampton, IT Innovation- Electronics and Computer Science, Southampton, United Kingdom

Study question: What is the optimal follicle tracking ultrasound strategy for controlled ovarian stimulation (COS) in order to minimise face-to-face interactions but retain predictive power for both trigger timing and over response?

Summary answer: Data from follicle tracking scans on day 8, 9 or 10 can be used to make accurate predictions of trigger timing and risk of over-response.

What is known already: British Fertility Society guidance for restarting ART following Covid-19 pandemic related shutdowns recommends aiming to reduce the number of visits for monitoring during ovarian stimulation. If reduction is needed, prioritising attendance for scans on the most predictive cycle days is prudent. Current evidence on optimal monitoring during ovarian stimulation is sparse, and protocols vary significantly, with some centres priding themselves on monitoring intensity. Small studies of simplifying IVF therapy by minimising monitoring have reported no adverse effects. There are opportunities to learn from the adaptations necessary during these extraordinary times to improve efficiency of IVF care in the longer term.

Study design, size, duration: A retrospective database analysis of 9294 ultrasound scans performed during monitoring of 2322 IVF cycles undertaken by 1875 women in a single centre. The primary objective was to identify when in the IVF cycle data obtained from ultrasound is most predictive of both oocyte maturation trigger timing and an over-response to stimulation. Ethical approval for this study was obtained from the University of Southampton ERGO II and NHS REC (IRAS Project ID: 275218).

Participants/materials, setting, methods: Anonymised retrospective database analysis of IVF/ICSI cycles at a tertiary referral IVF centre in the United Kingdom. Machine learning models built combining demographic and follicular tracking data to predict oocyte maturation trigger timing and over-response. The primary outcome was the cycle days from which scan data yields optimal model prediction performance statistics. Random Forest Regressors were implemented as our predictive models, trained using cross-validation at treatment cycle level and evaluated on the out-of-fold samples.

Main results and the role of chance: The earliest day for which our model has high accuracy to predict both trigger day and risk of over-response (>18 follicles >=11mm or >=18 eggs) is cycle day 8. The model using day 8 data is strongly predictive of the day of trigger administration MSE (mean squared error) 1.70 +/-0.11. At cycle day 8, our model can predict over-response with high precision and recall (AUROC of 0.91 +/- 0.01). The model for predicting trigger day uses patient age, number of follicles at baseline scan and follicle count by size for the current scan. The model to predict over-response uses age and number of follicles of a given size.

In contrast, models using data from a baseline scan are not highly predictive of the trigger administration day (the mean MSE of models using data only from baseline scans is 3.83 +/- 0.24). Over-response can be predicted at baseline using AFC and age with moderate accuracy (mean AUROC 0.77 +/- 0.01).

These results suggest that, if there is a significant need to reduce patient contacts per cycle in order to facilitate safe access to care, priority for patients attending for scans after day 7 of the treatment cycle should be considered.

Limitations, reasons for caution: This is a single-centre retrospective study. RCTs with healthy live birth primary outcome should guide changes to IVF care. However, for safety in extraordinary times, clinics may have to adjust protocols immediately, with no time to await optimal evidence. These results may provide help for those making these challenging decisions.

Wider implications of the findings: These results are timely and prospective evaluation of streamlined follicular tracking for COS monitoring may be warranted. Previous small studies have shown that minimal monitoring protocols did not adversely impact outcomes. If IVF can safely be made less onerous, without compromising success, this could help reduce burden related treatment drop-out.

O-316 Maternal-fetal vertical SAR-CoV-2 (COVID-19) viral transmission during pregnancies is possible and currently cannot be dismissed

G. Bahadur[1,2], R. Homburg[2], C. Singh[3], M. Bhat[4], P.A. Doreski[5], J. Huirne[6], A. Muneer[7]

[1]North Middlesex University Hospital Trust, Reproductive Medicine Unit, London, United Kingdom

[2]Homerton University Hospital, Homerton Fertility Centre-, - London E9 6SR, United Kingdom

[3]North Middlesex University, Neonatal and Peadiatric Unit-3, London N18 1QX, United Kingdom

[4]University Hospital Crosshouse-, Ayrshire Fertility Unit-, Kilmarnock- KA2 0BE-Scotland, United Kingdom

[5]FundacionRespirar-, Pulmonologist- Head of Clinical Research-, Av Cabildo 1548 1st A. Buenos Aires., Argentina

[6]University medical centers Amsterdam- location VUmc and AMC-, Research institute Reproduction and development-, De Boelelaan 1081- 1081 HV Amsterdam, The Netherlands

[7]University College London Hospital Trust-, NIHR Biomedical Research Centre-, 250 Euston Road. London NW1 2PG, United Kingdom

Study question: Is there vertical transmission (from woman to baby antenatally or intrapartum) after SARS-CoV-2 (COVID-19) infected pregnancy?

Summary answer: SARS-CoV-2 (COVID-19) infected pregnancies leaves open the possibility of vertical viral transmission from mother to baby.

What is known already: The majority of viruses do not cross the placental barrier; but when they do, can cause serious fetal illness including birth defects, miscarriage, abnormalities of growth and development, neurological injuries, fetal demise, preterm delivery and neonatal complications. Such examples are Zika, CMV, rubella, HIV/AIDS viral infections in pregnancy. The expression of SARS-CoV-2 receptors angiotensin-converting enzyme 2 (ACE2) plays essential roles in human infection and transmission and is found in maternal-fetal interface and human fetal heart, liver and lung. SARS-CoV-2 vertical transmission from mother to baby constitutes a major defining question in this pandemic to help clinical practices and patients.

Study design, size, duration: A systematic review searching terms related to SARS-CoV-2 (COVID-19), pregnancy, neonatal complications, viral and vertical transmission was suggested. The final computerized database search was performed on 30/04/2020. No language restriction was placed on published articles. The duration was from December 2019- 15th May 2020 for extracting the literature and screening the articles for potential interest. As data source was international there was considerable heterogeneity in patient exposures, quality control and possibly variants of SARS-CoV-2 virus.

Participants/materials, setting, methods: A systematic review was performed in PUBMED, EMBASE, CENTRAL, WEB of SCIENCE, Web of

Knowledge, the WHO, RCOG, ESHRE, ASRM, NEJM, BMJ, Lancet, Welcome and Cochrane Central Register of Studies, UKOSS, Office of National Statistics (ONS-UK) , Department of Health (UK), Google Scholar and any references of relevant articles. Data relevant to SARS-CoV-2 diagnostic interpretation was also collected. Mostly small case series with a high degree of heterogeneity was assembled on an excel spread sheet.

Main results and the role of chance: From 58 papers, 480 COVID-19 positive pregnant women were identified and 688 babies born covered in 80 publications. Most publications (47/80, 58.7%), reported small number (<5) of babies. From 71 papers, 10 babies were COVID-19 positive. 1st RT-PCR diagnostic tests were done in 356 babies, 2nd RT-PCR was done in 90 cases, IgM tests done in 28 babies, and IgG tests done in 28 babies. From 71 studies and 356 babies, 13 tested positive in 1st RT-PCR. After 2nd RT-PCR, 7 became negative, whereas 3 babies testing negative on 1st RT-PCR became positive. On 2nd RT-PCR, 3 babies became positive (who were initially reported as negative in 1st RT-PCR), 3 continued to remain positive (positive in 1st RT-PCR) but 3 who were positive in 1st RT-PCR were not tested again. On the 1st RT-PCR, 44 studies reported time of testing while 27 studies did not. Earliest RT-PCR positive test was 2 hours while in 2 studies, 10 women tested had positive amniotic fluid, and 3/11 placental swabs tested positive for SARS-CoV-2 RNA but babies remained negative. Changes to placental pathology reported.

In 4 unrelated report, 100% of patients initially tested negative, turning positive after 2nd and 3rd re-tests for SARS-CoV-2 RNA.

Limitations, reasons for caution: Caution should be exercised in interpreting test results; sampling methods, timing, patient infectivity level, SARS-COV-2 viability tests, sensitivity and specificity. Controls are generally lacking in reports. Small, globally heterogeneous cases reported. Voluntary case reports submission to research databases are heterogeneous, potentially breaching patient anonymity and some clinics may withhold data.

Wider implications of the findings: When factoring in the incubation period and efficacy of the diagnostic test results, it suggests the viral infection pre-existed the tests. Thereby it is likely that vertical transmission had occurred but validation and controlled experiments are needed to allow a definitive assessment to counsel pregnant women on SARS-COV-2 pandemic risks.

INVITED SESSION

SESSION 37: MHR SYMPOSIUM - FUNDAMENTALS ON MAKING OOCYTES

07 July 2020 Parallel 2 11:45 - 12:45

O-148 Major molecular players in creating oocytes from germ cells

J. Bowles[1]

[1]*University of Queensland, Australia*

O-149 Influence of genetic and maternal factors on the egg epigenome

G. Kelsey[1], **A. Galvao**[1], **J. Castillo-Fernandez**[1], **H. Demond**[1], **E. Herrera**[1]

[1]*Babraham Institute, Epigenetics Programme, Cambridge, United Kingdom*

Abstract text

Over the life course, the mammalian genome undergoes profound setting and resetting of epigenetic information. Germ-cell specification, gametogenesis, and early embryo development are characterised by phases of widespread erasure and rewriting of DNA methylation. This extensive reprogramming largely prevents transmission of epigenetic information across generations; however, reprogramming events must also ensure correct genomic imprinting, an essential epigenetic mechanism that does depend on faithful, long-term persistence of gamete-derived methylation in the next generation. This underscores the importance of understanding how methylation patterns are established in the germline and the extent to which they may be influenced by adverse physiological or environmental factors, or procedures applied in assisted reproduction.

We recently pioneered genome-wide analysis of DNA methylation in single cells, as well as methods for joint profiling of DNA methylation, gene expression and chromatin accessibility. We are deploying these capabilities to explore effects of factors such as maternal age and maternal diet on the epigenetic quality of the oocyte, and to investigate whether any anomalies detected persist in the embryo. In addition, with detailed knowledge of the mechanisms underlying DNA methylation in the oocyte, we are keen understand how such epigenetic anomalies arise.

INVITED SESSION

SESSION 38: LABORATORY SESSION - TIME-LAPSE IN 2020

07 July 2020 Parallel 3 11:45 - 12:45

O-150 Time-lapse technology: Lights and shadows of clinical evidence

A. Ahlstrom[1]

[1]*Livio Fertilitetscentrum Gothenburg, IVF Laboratory, Sweden*

O-151 How TLT is changing our understanding and research on embryo development?

T. Freour[1]

[1]*CHU de Nantes, Médecine de la Reproduction, France*

SELECTED ORAL COMMUNICATIONS

SESSION 39: STRATEGIES TO IMPROVE THE OUTCOMES OF OVARIAN STIMULATION 2

07 July 2020 Parallel 4 11:45 - 12:55

O-152 Impact of different strategies in frozen cycles in normo responding patients undergoing IVF/ICSI cycles: a multicenter cohort study

C. Siristatidis[1], **T. Arkoulis**[2], **N. Christoforidis**[3], **G. Salamalekis**[4], **N. Koutlaki**[5], **V. Karageorgiou**[6], **G. Galazios**[5]

[1]*University of Athens, 3rd Department of Obstetrics and Gynecology, Athens, Greece ;*
[2]*Mitosis IVF Clinic, Private IVF Clinic, Piraeus, Greece ;*
[3]*Assisted Reproduction Unit- Embryolab, Private Clinic, Thessaloniki, Greece ;*
[4]*Medical School- National and Kapodistrian University of Athens, Assisted Reproduction Unit- Third Department of Obstetrics and Gynecology- "Attikon Hospital", Athens, Greece ;*
[5]*Medical School- Democritus University of Thrace, Assisted Reproduction Unit- Department of Obstetrics and Gynecology, Alexandroupolis, Greece ;*
[6]*Medical School- National and Kapodistrian University of Athens, Medical School- National and Kapodistrian University of Athens, Athens, Greece*

Study question: Do different modalities used for frozen-thawed embryo transfer (FET) treatment cycles in normoovulatory patients undergoing IVF/ICSI are linked with different pregnancy rates?

Summary answer: Live birth was superior in HR cycles without GnRH analogs and miscarriage was higher when progesterone was administered through gel vs. tablets.

What is known already: A variety of strategies and regimens for FET have been used and proposed. Reports have been plenty, with no existing consensus regarding the best protocol to choose or to prepare the endometrium, through a luteal plase "creation" or replacement. Crucial parameters for the right choice consist of control and flexibility in the timing of transfer, cost, and proper endometrial development, along with rates of pregnancy, miscarriage and live birth. Currently, uncertainty exists concerning the type, adjunct or dose of regimen to offer, while many IVF Units use variable and mixed protocols.

Study design, size, duration: This is a four-center two-arm retrospective cohort study conducted at both University and Private Assisted Reproductive Units in Greece, using data from 456 cycles with vitrification from 369 women during the last 5 years.
Participants/materials, setting, methods: The following modalities were analyzed: 1. Natural cycle (NC), using hCG as ovulatory trigger, without luteal support (Group 1), 2. NC, using hCG, with luteal support (Group 2) 3. Hormone Replacement cycle (HRC) plus GnRHa suppression (Group 3) 4. HRC without GnRHa suppression (Group 4). The primary outcome measures were live birth and miscarriage rates.
Main results and the role of chance: From all four centers, data for 456 cycles through vitrification from 369 women was provided. Live birth was higher in Groups 2 and 3 as compared to Group 4 (by 81%), and when the stable estrogen dose was administered compared to the increasing, by 8.82 times (1.25, 62.03). Regarding miscarriage, 19 events were recorded; progesterone administration through gel was significantly associated with a higher risk, compared to tabs [4.77 (1.80, 12.67)]. Regarding secondary outcomes, biochemical and clinical pregnancy rates were lower in Group 3 compared to Group 4 [0.70 (0.6, 0.82) and 0.70 (0.61, 0.80), respectively] and when the stable estrogen dose was administered compared to the increasing [0.83 (0.72, 0.96) and 0.87 (0.77, 0.97), respectively]. The rest of the comparisons yielded non-significant results. In the multivariable analysis, live birth appeared to be significantly affected only by the endometrial preparation modality: Group 3 experienced higher rates compared to Group 4 (6.51 (2.66, 15.93)].
Limitations, reasons for caution: Limitations of the study include its retrospective nature that is linked with known and unknown biases and the small cohort size.
Wider implications of the findings: HRC without GnRHa and probably NC with hCG and luteal support appears to be superior to HRC with GnRHa, concerning live birth. Miscarriage was increased in women receiving progesterone gel compared to tabs. Age was a significant predictor of negative biochemical and clinical pregnancy rates.
Trial registration number: NCT03965949

O-153 Follicular flushing leads to higher oocyte yield in monofollicular natural cycle IVF – a randomized controlled trial

A. Kohl Schwartz[1], I. Calzaferri[1], M. Roumet[2], A. Limacher[2], A. Fink[1], A. Wueest[1], S. Weidlinger[1], V. Mitter[1], B. Leeners[3], M. Von Wolff[1]

[1]Bern University Hospital, Division of Gynecological Endocrinology and Reproductive Medicine, Bern, Switzerland ;
[2]Clinical Trial Unit Bern, University of Bern, Bern, Switzerland ;
[3]University Hospital Zurich, Department of Reproductive Endocrinology, Zurich, Switzerland

Study question: Does follicular flushing increase the number of mature oocytes in monofollicular natural cycle in-vitro fertilization?
Summary answer: Follicular flushing increases the number of mature oocytes, increases the fertilisation rate and reveals a trend towards a higher transfer rate.
What is known already: In polyfollicular IVF and in oligofollicular poor responders, flushing neither increases the oocyte yield nor the pregnancy rate. In monofollicular IVF the effect of flushing had so far been addressed by two studies. One prospective study with minimal stimulation IVF demonstrated an increased oocyte yield. One retrospective study with Natural Cycle (NC)-IVF showed an increased oocyte yield and an increase of the transfer rate. A prospective randomized study specifically analysing monofollicular IVF was still missing.
Study design, size, duration: Prospective randomized controlled trial including 164 women who were randomized for either aspiration without or with flushing from 2016-2019.
Participants/materials, setting, methods: Infertile women 18-42 years of age with an indication for IVF treatment at a university-based infertility unit. Women undergoing monofollicular NC-IVF in vitro fertilization were randomized to either follicular aspiration only or follicular aspiration directly followed by five follicular flushes at a 1:1 ratio. The intervention was done without anaesthesia, using a gauge 19 single lumen needle. Flushing volume was calculated (sphere formula) based on the size of the follicle.
Main results and the role of chance: A total of 164 women were included; 81 were allocated to "aspiration only," and 83 to additional "flushing." Primary analysis was based on the intention to treat: oocyte yield, defined as collected mature oocyte rate, was higher (n=64/83, 77.1%) in the flushing group compared to the aspiration only group (n=48/81, 49.3%, risk difference (RD): 18.2% (95% CI 3.8 - 31.6%), p-value=0.02). In the flushing group, most oocytes were retrieved in the first 3 flushes (63/83, 75.8%). Fertilization rate was higher in the flushing group (n=53/83, 63.9% versus n=38/81, 46.9%; RD: 16.8% (96% CI 1.5 - 31.4%), p= 0.045). Transfer rate was also higher in the flushing group (n=52/83, 62.7% versus n=38/81, 46.9%; RD: 15.71 (95% CI 0.3 - 30.3%), but the difference was not significant (p= 0.06). The clinical pregnancy rate per transfer was not significantly different between the two groups. The median duration of the intervention was significantly shorter with "aspiration only" (0.43 min; IQR 0.3, 0.5) versus "flushing" (2.38 min; IQR 2.0, 2.7; p<0.001). There was no significant difference in the mean VAS pain score between the follicular flushing (3.4 ±1.8) and the aspiration group (3.1 ±1.89).
Limitations, reasons for caution: Sample size was not powered to analyse pregnancy and live birth rates.
Wider implications of the findings: Our study proved that flushing of single follicles in Natural Cycle IVF increases the oocyte yield. In contrast to polyfollicular and oligofollicular IVF flushing seems to be beneficial in the monofollicular setting if the technique used in our study (single-lumen needle, 5 flushings with flushing volume adaptation) is applied.
Trial registration number: NCT 02641808.

O-154 Do we trust in evidence based medicine? A multicentre retrospective analysis of 2677 first IVF/ICSI cycles before and after the OPTIMIST trial.

E. Papaleo[1], A. Revelli[2], M. Costa[3], M. Bertoli[4], S. Zaffagnini[5], F. Tomei[6], F. Cantatore[1], M. Reschini[7], A. Rebecchi[1], F. Parissone[5], M. Manno[6], M. Sironi[4], T. Tessari[4], D. Colia[3], E. Somigliana[7]

[1]S. Raffaele Hospital, Centro Scienze della Natalità, Milano, Italy ;
[2]University of Turin, IVF unit, Turin, Italy ;
[3]International Evangelic Hospital, IVF Unit, Genoa, Italy ;
[4]Azienda Ospedaliera "C. Poma", IVF Unit, Mantua, Italy ;
[5]Azienda Ospedaliera Universitaria Integrata, IVF Unit, Verona, Italy ;
[6]Ospedale Santa Maria degli Angeli, IVF Unit, Pordenone, Italy ;
[7]Fondazione IRCCS Ca' Granda- Ospedale Maggiore Policlinico, IVF Unit, Milan, Italy

Study question: To evaluate whether the practice of individualizing r-FSH starting dose has been substituted and outdated after the largest RCT in ART, the OPTIMIST trial.
Summary answer: OPTMIST trial influenced physicians to standardize r-FSH starting dose and to reduce the mean starting dose in predicted POR women undergoing COS.
What is known already: Individualizing r-FSH starting dose has been subject of many studies over the past 20 years. Although the "one size fits all" approach has been discouraged for decades by most authors, van Tilborg *et al*. (2017) on behalf of the OPTIMIST study group demonstrated in a large prospective RCT that dosage individualization, in general, does not appear to improve overall live birth rates. This implies that, while reduction of dosage may create better safety in predicted high responders, the use of high dosages of r-FSH, typical of predicted low responders, lacks any cost-benefit advantage and may be abandoned.
Study design, size, duration: We performed a retrospective analysis of seven italian fertility centers including all first IVF/ICSI cycles from January 1st 2017 to December 31st 2018, before and after the OPTMIST publication on November 2017. Patients were distributed according to their ovarian reserve markers in: predicted POR (AFC ≤ 7 and/or AMH <1.1), predicted normo-responders (AFC 8-15 and/or AMH 1.1-3.5) and expected hyper-responders (AFC > 16 and/or AMH > 3.5).
Participants/materials, setting, methods: 2677 patients between 18 and 42 years old undergoing their first IVF-ICSI cycle were included. The study group A (before OPTIMIST) included 1325 patients who underwent their first cycle from January 2017 to December 2017. The study group B (after OPTIMIST) included 1352 patients who underwent their first cycle from January 2018 to December 2018. In both study groups data regarding patients characteristics (age, BMI, AMH, AFC,), stimulation protocols and stimulation outcomes were collected.

Main results and the role of chance: In the study group "before" 401/1325 (30, 2%) patients were expected POR, 566 (42, 7%) expected normo-responders, 358/1325 (27, 0%) predicted hyper-responders. In the study group "after" patients' distribution was 366/1352 (27.0%), 601/1352 (44.4%), 385/1352 (28.4%) for each group **(**p=NS**)**. Comparing 2017 and 2018 there was an increased prescription of standard 150UI proposed by OPTIMIST trial, from 20.8% to 24.1% (p=0.046). Interestingly, we also observed a significant reduction of overall use of starting dose >300UI from 10.3% in 2017 to 5.6% in 2018 (p <0.01). In predicted POR undergoing their first COS, the mean FSH starting dose reduced from 317UI to 302 UI (p< 0.03). This approach did not determine any differences in terms of oocytes retrieved in the before and the after group respectively (4.21 ± 3.1 vs 4.22 ±3.4, p=0.258), metaphase II oocytes when ICSI was used (3.04± 2.4 vs 3.2 ± 2.7, p=0.43) or total number of oocytes fertilized (IVF/ICSI) (3.0 ±2.6 vs 2.8 ± 2.59, p=0.42). Cumulative pregnancy rate was also comparable between the two years (20.3% vs 20.9% in 2017 and in 2018 respectively, p=0.84).

Limitations, reasons for caution: Retrospective nature of the study. Overall cumulative pregnancy rate is still partly pending at the time of data collection, because a proportion of non-pregnant patients (mainly hyper- and normo-responders and mainly treated in 2018) might return in the future for frozen embryo transfer attempts.

Wider implications of the findings: Our clinical results confirm that EBM provided by the OPTIMIST trial has somehow influenced our ART daily practice, bringing to a reduction of starting doses without limiting outcomes. On the other hand, adherence to the OPTIMIST indications is still generally poor.

Trial registration number: not applicable

O-155 Experience in random-start ovarian stimulation in cancer patients

Y. Martirosyan[1], T. Nazarenko, Alekseevna[2], A. Birukova, Mikhailovna[1], L. Dzhanashvili , Georgievna[1]

[1]National Medical Research Center for Obstetrics- Gynecology and Perinatology named after V.I. Kulakov- Ministry of Health of Russia, Research and educational center for assisted reproductive technologies named after F. Paulsen-senior, Moscow, Russia C ;

[2]National Medical Research Center for Obstetrics- Gynecology and Perinatology named after V.I. Kulakov- Ministry of Health of Russia, the Research and educational center for assisted reproductive technologies named after F. Paulsen-senior, Moscow, Russ

Study question: What is the effectiveness of ovarian function stimulation in the luteal phase of the cycle compared with the follicular phase in patients underwent fertility preservation?

Summary answer: Ovarian stimulation in the luteal phase allows us to optimize strategies of ovarian stimulation in cancer patients without compromising the oocyte yield.

What is known already: The choice of ovarian stimulation protocol for cancer patients is based on a balance between two main factors: the limited time available for fertility preservation (FP) before the start of gonadotoxic therapy, and the need to obtain a sufficient number of suitable oocytes and embryos for cryopreservation. Despite data about comparable efficacy of the ovarian stimulation with GnRH-ant in the follicular phase of the cycle and stimulation in the luteal phase, many questions remain open.

Study design, size, duration: We performed a prospective observational study at the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after V.I. Kulakov from February 2019 to December 2019. A total of 160 women who had FP consultation prior to neoadjuvant chemotherapy were identified. 47 patients were enrolled for the study according to inclusion criteria.

Participants/materials, setting, methods: Depending on the phase of the menstrual cycle when ovarian stimulation was started, two groups of patients were identified: I - ovarian stimulation started in the early follicular (n=34) and II - luteal phase group (n=13).

We measured the number of retrieved oocytes and mature oocytes, stimulation duration, initial and total gonadotropin dose, the parameters of steroidogenesis. Differences were tested using a two-sided Student's t-test or a Pearson's Chi2 test, as appropriate.

Main results and the role of chance: All patients included in the study were comparable in age and AMH level (33.38 ± 3.73; 33.3 ± 5.47 years, p=0.967580 ; AMH - 3.0 ± 2.14 ng /ml 2,72±0,41 ng / ml, p=0.693440). Antral follicle count (AFC) were significantly lower in the II group 9,2±1,2; 5,8±1,14, p=0.046535). There were no differences in the mean number of oocytes retrieved in the follicular phase group, and luteal phase group (10,33 ±2,57 versus 11,62±1,64; p=0.676949). Similarly, no significant differences were observed in the number of M II occytes (6,9±1,14 versus 8,92± 0,94; p=0.179224), stimulation duration (12,6±0,6; 13,5±1,1, p=0.476761), initial (243,06 ± 12,96 IU versus 233,33 ± 20,73 IU, p=0.694155) and total gonadotropin dose (2635,44 ±187,75 IU versus 2145,83 ±277,72 IU, p=0.158947). Estradiol (E2) and progesterone (P) levels on the day when the ovulation stimulation was started were different among the two groups (211,62±40,19 pmol/L versus 693,47±151,04 pmol/L, p<0.05;1,13±0,1 nmol/L versus 22,44±9,97 nmol/L, p<0.05). Perhaps due to the regression of the corpus luteum, we observe comparable levels of E2 and P in both groups on the 6th day of ovarian stimulation and on the day of the trigger administration. Premature LH surge did`t observe in the absence of GnRH-ant administration in the II group.

Limitations, reasons for caution: The current work is a pilot study. Larger prospective trials are planning.

Wider implications of the findings: Our data allow us to talk about the comparable effectiveness of ovarian stimulation of the follicular and luteal phases of the cycle. Modern knowledge on folliculogenesis lets us to optimize strategies of ovarian stimulation not only from the point of flexibility.

Trial registration number: None

O-156 Pituitary supression is not necessary for blocking LH surge during luteal-phase stimulation

M. Cruz Palomino[1], E. Henzenn[1], A. Requena[1]

[1]IVI Madrid, Reproductive Medicine, Madrid, Spain

Study question: Can we avoid the administration of pituitary suppressors during a luteal phase stimulation without affecting the ovarian response?

Summary answer: In absence of pituitary supressors during luteal-phase stimulation, it is posible to block a physiological LH surge without impacting normal ovarian response

What is known already: New stimulation approaches allow for a total disarticulation between the time of the menstrual cycle, ovarian stimulation start and embryo transfer. Double stimulation (DuoStim) was initially designed to optimize clinical outcomes in poor ovarian response, but it could be also useful in fertility preservation for non-medical reasons, especially for oocyte/embryo accumulation. Pituitary suppressors block the LH surge; however, in a protocol as specific as DuoStim, it could be assumed that these suppressors are not necessary in the luteal phase because the endogenous progesterone released during follicular phase is sufficient to block the LH surge during the luteal phase

Study design, size, duration: Prospective and observational analysis performed in IVI Madrid between September and December 2019. Participants were randomly assigned to each of the study groups. Participants underwent the same stimulation protocol in the follicular phase and for luteal phase stimulation, they were allocated in a control group with pituitary suppressors (n=10) or in a study group, where this medication was not administered (n=10). Statistical analysis was performed by ANOVA and Chi-squared where applicable.

Participants/materials, setting, methods: Follicular-phase stimulation was the same for both study groups; daily tablet of 10 mg of acetate of medroxyprogesterone (AMP) from first day of stimulation, 225 IU/day ecombinant FSH and triggering with 0.1 mg GnRH agonist. For the control group, luteal-stimulation is identical to the previous one; and in the study group, the only difference is that AMP was not administered daily from the start of the stimulation. LH, estradiol and progesterone were monitored during luteal-phase.

Main results and the role of chance: As expected, and in the case of a homogeneous population such as oocyte donors, no differences were observed between the two study groups in follicular-phase stimulation, either with respect to endocrine profile or ovarian response. For control and study group respectively, the results were as follows: basal LH (6.11±1.6 IU vs 6.6±2.4 IU, p=0.680); LH on the triggering day (3.1±1.8 IU vs 2.3±0.7 IU, p=0.548); progesterone on the triggering day (1.4±0.3 ng/ml vs. 1.1±0.1 ng/ml, p=0.180); retrieved oocytes (16.7±3.0 vs. 20.1±5.4, p=0.389);and metaphase II oocytes (14.2±3.5 vs. 16.7±4.5, p=0.496).

These results are maintained for the luteal-phase stimulation, meaning that the endogenous profile in the stimulated folicular-phase is capable of inhibiting LH surge, not affecting the results derived from this second stimulation. For control and study group respectively, the results were as follows: basal LH (1.6±1.3 IU vs 1.7±0.6 IU, p=0.335); LH on the triggering day (0.5±0.4 IU vs 1.5±0.6 IU, p=0.300); progesterone on the triggering day (0.6±0.1 ng/ml vs. 0.4±0.1 ng/ml, p=0.398); retrieved oocytes (14.5±1.6 vs. 12.8±2.1, p=0.575);and metaphase II oocytes (11.7±1.0 vs. 10.4±2.3, p=0.609).

Finally, it should be noted that no rescue protocol with administration of GnRH antagonist was applied in the study group, because of ovulation risk

Limitations, reasons for caution: These results could be considered as an interim analysis, as they are framed within a pilot study prior to conducting a larger study so, although the current data are encouraging, we are not able to draw solid evidences due to our small simple size

Wider implications of the findings: In a certain group of patients such as oocyte donors, double stimulation implies advantages such as the possibility of achieving more oocytes in less time, optimizing the economic profitability of the egg donation program without compromising clinical results becuase of the absence of pituitary supresor during luteal-phase.

Trial registration number: Not applicable

INVITED SESSION

SESSION 40: GLOBAL ART MONITORING

07 July 2020 Parallel 2 14:00 - 14:45

O-157 ICMART preliminary world report 2016

G.D. Adamson[1], S. Dyer[2], G. Chambers[3], O. Ishihara[4], R. Mansour[5], M. Banker[6], J. de Mouzon[7], M. Kupka[8], F. Zegers-Hochschild[9]

[1]Stanford University School of Medicine, Reproductive Endocrinology and Infertility, Palo Alto, U.S.A.
[2]Groote Schuur Hospital and Faculty of Health Sciences, Reproductive Medicine Unit, Cape Town, South Africa
[3]University of New South Wales, School of Women's and Children's Health SWCH and Centre for Big Data Research in Health CBDRH, Sydney, Australia
[4]Saitama Medical University, Department of Obstetrics and Gynecology, Saitama, Japan
[5]The Egyptian IVF-ET Center, Reproductive Medicine, Cairo, Egypt
[6]Pulse Women's Hospital, Reproductive Medicine, Ahmedabad Gujarat, India
[7]Inserm, Reproductive Medicine, Paris, France
[8]Ludwig-Maximilians-University, Reproductive Medicine, Munich, Germany
[9]Universidad Diego Portales, Program of Ethics and Public Policies in Human Reproduction, Santiago, Chile

Abstract text

Abstract title: International Committee for Monitoring Assisted Reproductive Technologies (ICMART) Preliminary World Report on ART, 2016

Study question: In 2016 what was global utilization, effectiveness and safety of ART?

Summary answer: Globally, ART utilization and data collection continue to increase but with wide variations in utilization, effectiveness and safety.

What is known already: ICMART began ART global data collection in 1991. Utilization, effectiveness and safety have continuously improved with more cycles, higher pregnancy rates and lower multiple birth rates, the latter as a result of transfer of fewer embryos. Frozen embryo transfer (FET) and donor egg cycles continue to increase. However, wide variations in practice and outcomes exist globally. Over 8 million ART babies have been born. ICMART has helped develop registries internationally, notably with the African Network and Registry for Assisted Reproductive Technology (ANARA). A new electronic data collection platform is being developed; nevertheless, data collection and quality remain challenging. China has recently published its first comprehensive report on assisted reproductive technology service availability, efficacy and safety in mainland China: 2016.

Study design, size, duration: Countries and regions annually collect ART data, some prospectively and others retrospectively. ICMART retrospectively requested these data from all known global sources for 2016 and reviewed them for missing or incorrect data. The dataset was corrected and then analyzed utilizing standardized definitions from The ICMART/WHO Revised Glossary on ART Terminology, 2009 which was current at the time but is now replaced by The International Glossary on Infertility and Fertility Care, 2017, and previously developed methods. Preliminary results are presented. China results are summarized and compared with other global data.

Participants/materials, setting, methods: The European IVF Monitoring Consortium (EIM), Latin American Network of Assisted Reproduction (REDLARA), Australian/ New Zealand Registry and ANARA submitted regional data, and other countries contributed national data, through standardized formats to ICMART. A few individual clinics with no registry access also contributed. Data were reviewed, corrected, validated to the extent possible, analyzed and summarized by ICMART using descriptive statistics. China CDC and National Health Commission published their data in Human Reproduction.

Main results and the role of chance: Data collection and analysis are ongoing, so presented results are preliminary. The number of ART cycles continues to increase, but utilization is still highly variable among countries and regions. Regional and country differences persist in the age of the population treated, number of embryos transferred, rate of multiple births, use of ICSI, cryopreservation cycles and other factors.

The role of chance is minimal. Actual global ART results are limited to reporting countries and clinics representing approximately 2/3 of global cycles. However, this is a very large sample size from which imputation of total global results is performed. Addition of data from China improves significantly global reporting of ART data. China results are not different than overall global data.

Limitations, reasons for caution: Some countries have limited data and many countries have limited data validation. ICMART can perform only minimal verification of submitted data. Widespread adherence to consensus definitions provided in the Glossary takes time and requires translation into multiple languages. China data has some similar and different limitations.

Wider implications of the findings: ICMART World Reports standardize data, track trends, enable comparisons, stimulate questions and improve ART quality. Better understanding of ART increases societal acceptance and support for equitable access and ART research. Addition of China data is an important milestone in global ART data reporting.

Trial registration number:

Study funding:

Funding source:

O-158 SET in a global perspective. Regional similarities and differences

O. Ishihara[1], F. Zegers-Hochschild[2], J. de Mouzon[3], S. Dyer[4], R. Mansour[5], M. Banker[6], G. Chambers[7], D. Adamson[8]

[1]Saitama Medical University, Department of Obstetrics & Gynaecology, Iruma-gun- Saitama, Japan
[2]University Diego Portales, Clinica las Condes and Program of Ethics and Public Policies in Human Reproduction, Santiago, Chile
[3]Epidémiology- research and development, Paris, France
[4]Groote Schuur Hospital and Faculty of Health Sciences- University of Cape Town, Department of Obstetrics & Gynaecology, Cape Town, South Africa
[5]Egyptian IVF-ET Center, Egyptian IVF-ET Center, Cairo, Egypt
[6]Nova IVI Fertility, Nova IVI Fertility, Ahmedabad, India
[7]University of New South Wales, National Perinatal Epidemiology and Statistics Unit- Centre for Big Data Research in Health- School of Women's and Children's Health, Sydney, Australia
[8]Equal3 Fertility, Equal3 Fertility, Cupertino, U.S.A.

Study question: How is the transition to SET being realized in a global perspective? What are key issues for policymaking regarding the number of embryos transferred in various countries in the world?

Summary answer: The global SET rate significantly rose during the last 10 years, resulting in safer ART outcomes with lower multiple pregnancy rates and better infant indicators. However, different policies on SET resulted in some countries and regions continuing to have very high multiple rates. It is important to consider the use of multiple strategies that have successfully been introduced

in some countries to encourage the transition to SET and have resulted in safer ART.

What is known already: Decreasing the number of embryos transferred at ART strongly correlates with better outcomes of infants, namely, lower multiple pregnancy rates, lower preterm rates and fewer low birthweight infants. Acceptance of SET has been variable in regions and countries due to multiple factors.

Study design, size, duration: Retrospective analysis of the accumulated ICMART world ART registry data between 2002 and 2014 which had 601,243 and 1,647,777 initiated cycles reported, respectively, to determine the trend of average number of embryos transferred, the SET rates, and their outcome parameters by countries. Individual country data are also used for selected counties.

Participants/materials, setting, methods: ICMART world ART registry data annually reported by contributors from regional or national registries from 53-77 countries between 2002 and 2014.

Main results and the role of chance: The global average number of fresh embryos transferred and the SET rate were 2.47 and 12.4% in 2002 and improved to 1.73 and 40% in 2014. In FET cycles the results were even better at 1.43 and 61.6% in 2014. In response to the decreased number of embryos transferred, global multiple pregnancy rates in fresh non-donor IVF and ICSI cycles dropped from 28.2% (triplet 2.5%) in 2002 to 16.7% (triplet 0.5%) in 2014. However, countries can be grouped into 3 categories, those: 1) continuously maintaining highest SET rate; 2) achieving higher SET rate during the period; 3) continuing to have low SET rates throughout the period. The highest countries have SET rates of approximately 80% while other countries have only 10-20% with multiple pregnancy rates of approximately 30%.

Limitations, reasons for caution: The data accumulated by ICMART from different countries are of variable quality and comprehensiveness. Additionally, it is not possible for ICMART to validate the data.

Wider implications of the findings: These trends are important to follow. Clinical practices regarding number of embryos transferred are affected by cultural values, societal financial support of ART, changes in technology such as PGT-A, and other factors.

INVITED SESSION

SESSION 41: ALMER EXCHANGE SESSION - IVF LABORATORY AUTOMATION

07 July 2020 Parallel 3 14:00 - 15:00

O-159 Cons

C. Racowsky[1]

[1]*Brigham & Woman's Hospital, Department of Obstetrics & Gynecology ASB 1 + 3*

O-160 Pros

G.D. Smith[1]

[1]*6428 Medical Science I University of Michigan, Departments of OB/GYN- Physiology- and Urology, Ann Arbor- MI, U.S.A.*

Abstract text

Andrology and embryology laboratory practices have evolved over the last 4-5 decades, ultimately leading to increased technologies, increased personnel work hours per cycle, and increased success in treating infertility and preserving fertility. The manual labor component of andrology/embryology laboratory is intensive with many technologies requiring high degree of training and subject to technologist variability, drift, and retraining. Integration of new principles of bioengineering, microfluidics, and automation have been beneficial in other areas of everyday life, science, and medicine. In this presentation we will provide examples of scientific and social automation and discuss potential benefits. The integration of microfluidics for mechanical benefit and microenvironmental/physiological benefit will be proposed. Discussions will focus on proof-of-principle applications of microfluidics and automation to andrology, embryology, and cryobiology as they relate to laboratory tasks and the potential benefits they provide, both practically and biologically. Finally, we will discuss the goals and needs for integration of microfluidics for cell manipulation, insemination, culture, sampling, assays, and cryopreservation. This integration will facilitate automation within the clinical andrology/embryology laboratory and will have enabling influences on other evolving technologies such as machine learning and artificial intelligence for data collection and decision making in the IVF lab. These are not trivial goals and will require significant design, testing, and multidisciplinary interactions to be successful in the next generation of andrology/embryology practical laboratory patient care.

INVITED SESSION

SESSION 42: STRESS AND INFERTILITY - THE CHICKEN OR THE EGG

07 July 2020 Parallel 4 14:00 - 15:00

O-161 Just relax and it will happen?: the case for stress causing lack of pregnancy

J. Boivin[1]

[1]*Cardiff University, School of Psychology (Cardiff Fertility Studies Research Group), United Kingdom*

O-162 Just relax and it will happen?: The case against stress as a cause of infertility

A. Lawson[1]

[1]*Northwestern University, Obstetrics & Gynecology and Psychiatry, Chicago, U.S.A.*

Abstract text

"Just relax. It will happen." It's a phrase commonly heard by women struggling to get pregnant. Although the statement is likely intended to let women know that others are optimistic about their chances of having a baby, at its core it blames women for being too stressed to conceive. For generations, many have believed that psychological stress caused physiological changes that interfered with a woman's chances of conceiving. Although anecdotal stories of women relaxing and getting pregnant have been widely shared, rigorous research examining the role of stress in the etiology of infertility is limited. What little research exists does not support a direct link between the relationship between stress and infertility.

If, as hypothesized, psychological stress/distress can interfere with fertility then it could be argued that activities that produce a relaxation response could improve pregnancy chances. Indeed multiple studies have examined the positive effects of relaxation or relaxing activities and the cessation of stress inducing activities (e.g, fertility treatment) on both fertility and the reduction of emotional distress. However, these studies are also often not appropriately designed to rigorously answer the question about whether or not a relationship between relaxing and pregnancy chances exists. Because controversy exists regarding the relationship (or lack thereof) between stress and infertility, inconsistency in counseling of patients regarding the need for stress reduction is rampant. This presentation will address the biological and psychological issues in stress and infertility and will provide direction to providers for how to address these issues with patients.

SELECTED ORAL COMMUNICATIONS

SESSION 43: ICSI IN 2020

07 July 2020 Parallel 1 15:15 - 16:30

O-163 Advancement in the future automation of ICSI: Use of deep convolutional neural networks (CNN) to identify precise location to inject sperm in mature human oocytes

J. Dickinson[1], A. Meyer[1], N. Kelly[1], P. Thirumalaraju[2], M. Kanakasabapathy[2], D. Kartik[2], C. Bormann1, H. Shafiee[3]

[1]Massachuesetts General Hospital, Obstetrics and Gynecology, Boston, U.S.A. ;
[2]Brigham and Women's Hospital, Division of Engineering in Medicine- Department of Medicine, Boston, U.S.A. ;
[3]Brigham and Women's Hospital, Department of Medicine- Harvard Medical School, Boston, U.S.A.

Study question: Can a deep-learning artificial intelligence algorithm be used to accurately identify the appropriate location to perform ICSI on metaphase II (MII) human oocytes?
Summary answer: The AI trained network can be used to accurately identify the correct location on the oocyte to inject sperm for ICSI.
What is known already: ICSI is a procedure that includes, alignment of MII oocytes, selection and immobilization of sperm, and injection of sperm at a precise location that does not interfere with the mitotic spindle. The spindle is located adjacent to the extruded polar body (PB) and cannot be visualized using brightfield microscopy. Therefore, it is standard practice to align oocytes based on the location of the PB and to inject sperm 90° from this visible structure. The goal of this study was to train an artificial intelligence (AI) algorithm to identify the position of the PB and the corresponding location for sperm injection.
Study design, size, duration: Using a retrospective dataset of denuded MII oocytes, a deep CNN model was trained and tested to classify between 12 classes at 4-6 hours post oocyte retrieval. The twelve classifications resembled the pattern of digits on a clock, spaced 30° apart to provide an accurate location the extruded PB.
Participants/materials, setting, methods: We developed a deep convolutional neural network that was trained with 13992 annotated images of MII oocytes. We classified the location of the PB and corresponding location for sperm injection. The validation set containing 1920 oocyte images served to ensure the program training was complete. The developed network was evaluated using another independent set of 3900 MII oocyte images with known PB and sperm injection location classifications.
Main results and the role of chance: The deep learning CNN was able to correctly identify the location of the PB and corresponding location for sperm injection with 98.9% accuracy with a 95% confidence interval (CI) ranging between 98.5% to 99.2% (n=3900). Furthermore, a receiver operator characteristic (ROC) revealed micro and macro area under the curves (AUC) of 1, which confirmed that the AI can accurately identify the position of the PB and the corresponding location for sperm injection.
Limitations, reasons for caution: Only MII oocytes were used in this trial. Images were obtained using a single imaging platform (EmbryoScope) at a single timepoint. All cumulous cells were removed from the oocytes prior to training. It is unclear whether residual cumulous cells would affect the accuracy of the algorithm.
Wider implications of the findings: Advanced ICSI automation requires precision that cannot be achieved using tracking algorithms. Automation requires complex decisions that can be achieved using deep-learning technologies. Prior studies showed ability for CNNs to identify morphologically normal sperm. This study compliments this work by accurately identifying the correct position to inject sperm into oocytes.
Trial registration number: not applicable

O-164 The application of AOA-artificial oocyte activation in patients with previous unsuccessful attempt increases the ongoing pregnancy per treatment but not per transfer; a multivariable study

A. Galán Rivas[1], L. Alegre[1], M. Meseguer[1], T. Viloria[1], A. Pellicer[2], A. Tejera[1]

[1]Instituto Universitario IVI Valencia, IVF Lab, VALENCIA, Spain ;
[2]IVIRMA Roma, Reproductive Medicine, Roma, Italy

Study question: To check the effectiveness of AOA in patients with previous very low or failed fertilization rates in terms of fertilization, implantation and ongoing pregnancy outcomes.
Summary answer: After applying AOA the ongoing pregnancy rates of the patients per initiated treatment were enhanced, as well as a significant reduction in the cancellation rate.
What is known already: Different authors have demonstrated better results after using AOA in couples with low or non-fertilization in previous treatments: (Heindryckx et al., 2005, Heindryckx et al., 2008, Montag et al., 2012). Recently, we have seen extensively described similar benefits by the use of AOA for couples with previous standard ICSI failure (Fawzy et al., 2018). The implementation of this technique in the current clinical routine has been possible after reporting no detrimental impact on neither human gametes (Ebner et al., 2015) nor on the offspring (Vanden Meerschaut et al., 2014).
Study design, size, duration: Retrospective cohort study, from four consecutive years. We studied 509 oocytes from 66 patients who were treated with standard ICSI, and were compared to 616 oocytes from the same cohort of patients but using AOA.
Participants/materials, setting, methods: 66 patients were included in the study generating 163 cycles; standard group was composed of 509 oocytes generating 75 cycles (18 fresh cycles, 4 frozen cycles, 1 mixed and 52 cancelled cycles). AOA group included 616 oocytes resulting from the same patients, but creating 88 cycles (31 fresh cycles, 37 frozen cycles). AOA technique involves oocyte injection by spermatozoa and incubation for 10 minutes with calcium ionophore. Outcome analysis included a multivariable logistic regression model.
Main results and the role of chance: Although no differences were observed in relation with the day of transfer (Day 3 vs Day 5) and maternal age at the time of the cycle, we performed a multivariable logistic regression including those variables as potential bias factors. The study was performed to assess the impact of AOA on ongoing pregnancy per cycle as well as per transfer. We observed that the application of AOA in our patients increased the chances of a viable pregnancy by more than 4 times (OR=4.57, p=0.008) per cycle but not per transfer (OR=0.964, Not significant). When embryos were available for transfer AOA did not increased the chances of a viable pregnancy.
Limitations, reasons for caution: The retrospective analysis of this study may be a reason to take into consideration. Another limitation was not to study the PLCζ levels of the sperm, then we presumed that this dysfunction was presented in the spermatozoa and, in consequence, was the responsible for the fertilization failure.
Wider implications of the findings: Our findings suggest the use of AOA for a particular population where the fertilization was failed in previous attempts. After AOA application, the fertilization rate was enhanced, increasing the chances of success per treatment. The use of AOA is comforting after checking non-existence of detrimental impact on the offspring.
Trial registration number: none

O-165 Toward an ICSI chip: automated microfluidic oocyte denudation module

A. Mokhtare[1], P. Xie[2], A. Abbaspourrad[1], Z. Rosenwaks[2], G. Palermo[2]

[1]Cornell University, Department of Food Science and Technology, Ithaca, U.S.A. ;
[2]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: To determine whether novel microfluidic technologies can improve the efficiency and quality of an oocyte denudation process for intracytoplasmic sperm injection (ICSI).
Summary answer: The inevitable exposure of oocytes to high mechanical stresses, different oxygen concentrations, and temperature variations can be alleviated using an automated microfluidic oocyte denudation module.
What is known already: A microfluidic device has recently been introduced in the clinical field and has proven successful in selecting competent spermatozoa. Conventional cumulus removal (CR) techniques use high mechanical stress (pipette flushing) with enzymatic treatment or high fluidic stresses (vortexing) to treat cumulus-oocyte-complexes (COC) prior to ICSI. These operations not only impose unfavorable stresses on oocytes, but also require a highly skilled embryologist. Microfluidic technologies offer novel opportunities to streamline ART to facilitate gamete preparation, reduce mechanical and cytotoxic stress, and minimize inter-operator variability. Such automated and continuous systems

may replace manual CR operation while ensuring optimal environmental conditions.

Study design, size, duration: We developed a semi-automated oocyte denudation microfluidic chip (ODMC) controlled through microcontrollers with a graphical user interface (GUI). The chip consists of a 2-layer microchannel, with channel width similar to the diameter of mouse COC (200 µm). The bottom layer contains bas-relief structures to gently agitate the pressure-driven laminar flow inside microchannels without imposing excessive stress on the oocytes. Denudation rate and embryo developmental competence were compared between oocyte cleaning by ODMC and manual pipetting.

Participants/materials, setting, methods: COCs were retrieved from 3 superovulated B6D2F1 mouse oviducts and individually dissected. ODMCs were fabricated from polydimethylsiloxane (PDMS) by planar photolithography and installed with inlet and outlet reservoirs that facilitate injection and expulsion of hyaluronidase (80 IU/ml) throughout ODMCs. COCs were gently oscillated inside the channels on top of the bas-relief structures and denuded by laminar chaotic mixing. The GUI allows users to assess denuding oocytes in real-time and unload cleaned oocytes at their discretion.

Main results and the role of chance: Murine COCs were processed by ODMC with optimized geometry and operating parameters including oscillating frequency, flow rate, channel width, and number of bas-relief structures. The oscillating frequency and flow rate were able to tumble COCs to expose the entire surface to bas-relief structures to evenly strip-off cumulus cells (CCs). Using a high-speed camera recording real-time denudation, we observed that angular displacement of fluid and transverse flows surrounding COCs are the main denudation mechanisms. Computational fluid dynamics (CFD) simulations quantified microfluidic profiles in the ODMC to that of manual pipetting (MP). CFD results showed that the maximum shear stress imposed on COCs was 40% less in the ODMC compared to MP at the same flow rate. Denudation efficiency, defined as the percentage of complete CC deprivation at the outlet reservoir, was comparable between the ODMC and MP (95% vs. 91%) without oocyte loss. To ensure that our ODMC does not compromise the developmental potential of the gametes, piezo-actuated ICSI was performed on 50 oocytes processed by each method. ODMC and MP groups yielded comparable post-ICSI survival (84.0% vs. 82.0%), fertilization (92.9% vs. 90.2%), and blastocyst formation rates (90.5% vs. 87.8%), confirming that ODMC does not have adverse effects on preimplantation development.

Limitations, reasons for caution: PDMS is known for easy prototyping, high optical transparency, and biocompatibility. However, adverse effects including small molecule adsorption and gas permeability can be disadvantageous in delicate embryology procedures. Large-scale mouse embryo assay should be performed to assess teratogenicity of PDMS. Clinical-grade polystyrene may be utilized due to a lower toxicity.

Wider implications of the findings: This device offers a glimpse into the potential of a fully automated embryology laboratory and is being optimized for ICSI. Constructing different modules in addition to COC denudation is required for maturity recognition, injection, and storage of the conceptuses to follow embryo evaluation when applied in conjunction with artificial intelligence.

Trial registration number: not applicable

O-166 Marginal differences in preimplantation development between conventional IVF and ICSI in patients with non-male factor infertility: a sibling oocyte study.

N. De Munck[1], A. Bayram[1], A. Arnanz[1], A. Abdala[1], I. El-Khatib[1], A. El-Damen[1], L. Melado[2], B. Lawrenz[2], H.M. Fatemi[2]

[1]IVI RMA Middle East Fertility Clinic LLC, IVF lab, Abu Dhabi, United Arab Emirates ; [2]IVI RMA Middle East Fertility Clinic LLC, Gynaecology, Abu Dhabi, United Arab Emirates

Study question: Are there differences in the morphokinetic behavior between conventional IVF and ICSI in cycles with pre-implantation genetic testing for aneuploidies?

Summary answer: Preimplantation development is marginally, yet significantly different between embryos generated by conventional IVF and ICSI.

What is known already: Conventional IVF results in a delayed (4 hours) pronuclear formation and first mitotic divisions when compared to ICSI on sibling oocytes. This difference disappears around the time of morula formation in donor oocyte cycles. On the other hand, it has also been shown in autologous oocytes that IVF blastocysts start to expand 3 to 4 hours earlier than ICSI-generated blastocysts. When standardizing for the time of pronuclear fading, the differences in early cleavage disappear between IVF and ICSI, while blastulation and blastocyst expansion occurs earlier for IVF embryos.

Study design, size, duration: Prospective cohort study between November 2018 and April 2019, including 568 oocytes (30 patients) with non-male factor infertility for which half of the sibling oocytes were inseminated with conventional IVF (n=283) and the other half with ICSI (n=285). Embryos were cultured in an Embryoscope time-lapse incubator and trophectoderm biopsy was performed on good-quality blastocysts. The following timings were annotated: tPNf, t2-9, tSC, tM, tSB, tB, tEB, cc2(t3-t2), cc3(t5-t3), s2(t4-t3), s3(t8-t5) and Blast(tSB-t2).

Participants/materials, setting, methods: Univariate ($p<0.20$) and multivariate ($p<0.05$) analysis was performed in order to find morphokinetic differences between conventional IVF and ICSI. A secondary analysis was performed which corrects for the difference in time of fertilization, by standardizing all time lapse parameters for the time of pronuclear fading. Subgroup analysis for all day 5+6 biopsied blastocysts was performed. Results are presented as average ± SD, Odds Ratio [95% CI].

Main results and the role of chance: A total of 283 and 285 cumulus oocyte complexes were assigned to conventional IVF and ICSI of which 183 (64.7%) and 190 (66.7%) were normally fertilized, respectively. Conventional IVF generated 120 blastocysts that were biopsied, of which 59 (49.2%) were euploid, while 116 blastocysts were biopsied after ICSI of which 56 (48.3%) were euploid. Gender distribution (male/female) for blastocysts with informative outcome was 60/49 and 50/55, respectively.

When comparing the development of all normally fertilized zygotes between IVF and ICSI in the univariate model, a significant difference was found for tPNf ($p=0.005$), t2 ($p<0.001$), t3 ($p=0.001$), t4 ($p=0.003$), t5 ($p=0.001$), t6 ($p=0.096$), t7 ($p=0.177$) and Blast ($p=0.004$) of which only t2 remained significant in the multivariate model (OR: 1.282 [1.020-1.612], $p=0.033$); IVF: 29.3 ± 10.4 versus ICSI: 25.9 ± 5.1. After standardizing for tPNf, only corrected tSB ($p=0.009$) and Blast ($p=0.004$) showed significant differences between IVF and ICSI. However, only Blast appeared significant in the multivariate model: OR: 0.803 [0.648-0.994], $p=0.044$; IVF: 70.8 ± 8.2 versus ICSI: 72.5 ± 9.7. Taking into consideration the standardized kinetics of biopsied blastocysts, only a difference was observed for t2: OR: 1.519 [1.045-2.206], $p=0.028$; IVF: 3.4 ± 2.6 versus ICSI: 3.1 ± 3.3.

Limitations, reasons for caution: Only couples with non-male factor infertility and a normal response to ovarian stimulation were included, and therefore, the results cannot be extrapolated to other patient populations.

Wider implications of the findings: Following the recent debate on the over-use of ICSI, this study shows that conventional IVF results in the same number of blastocysts for biopsy with similar developmental kinetics, thereby reinforcing the use of conventional IVF in this patient population.

Trial registration number: NCT03708991

O-167 Intracytoplasmic sperm injection versus conventional in vitro fertilisation in couples with non-male factor infertility: a randomised controlled trial

Q.V. DANG[1], L. Vuong[2], Q. Nguyen[1], T. Ho[3], A. Ha[1], B. Truong[3], Q. Pham[4], R. Wang[5], R. Norman[6], B. Mol[5]

[1]My Duc Hospital, IVF Department, Ho Chi Minh City, Vietnam ; [2]University of Medicine and Pharmacy at Ho Chi Minh City, Department of Obstetrics and Gynecology, Ho Chi Minh City, Vietnam ; [3]An Sinh Hospital, IVF Department, Ho Chi Minh City, Vietnam ; [4]My Duc Hospital, HOPE Research Center, Ho Chi Minh City, Vietnam ; [5]Monash University, Department of Obstetrics and Gynecology, Melbourne, Australia ; [6]The University of Adelaide, Robinson Research Institute and Adelaide Medical School, Adelaide, Australia

Study question: Does intracytoplasmic sperm injection (ICSI) result in a higher ongoing pregnancy rate compared to conventional in vitro fertilisation (IVF) in couples with non-male factor infertility?

Summary answer: In couples with non-male factor infertility, ICSI did not result in a higher ongoing pregnancy rate compared to conventional IVF

What is known already: There is an increasing trend worldwide in the use of ICSI for couples with non-male factor infertility. Randomised controlled trials

(RCTs) have not demonstrated better outcomes of ICSI compared to conventional IVF, but their statistical power was limited and most studies used fertilisation as primary outcome. Moreover, non-randomised studies suggest no benefit and maybe even harm from ICSI compared to conventional IVF in this population.
Study design, size, duration: This open-label, multi-center RCT was conducted at two IVF centers in Vietnam. The protocol has been published (Dang et al., 2019). A sample size of 1064 couples was calculated to demonstrate a 10% difference between ICSI and conventional IVF (power 0.90, two-sided alpha 5%, loss to follow-up 10%). Randomisation was done using a computer-generated randomisation list, with a variable block size of 2, 4 or 8.
Participants/materials, setting, methods: Couples were eligible if the male partner had normal sperm count and motility (WHO, 2010) and had undergone ≤2 previous IVF/ICSI attempts. Women undergoing in vitro maturation cycles, using frozen semen or having a poor fertilisation (≤25%) in previous cycle were not eligible. The primary outcome was ongoing pregnancy resulting in live birth after the first embryo transfer. Intention-to-treat analyses were used.
Main results and the role of chance: Between March 2018 and August 2019, 1064 couples were randomly assigned to ICSI (n=532) or conventional IVF (n=532). Baseline characteristics were comparable between the two groups (mean female age 32 years, total sperm counts 190 million, sperm motility 43%). The follow-up for live birth is ongoing and will be available by June 2020. Ongoing pregnancy rates after the first embryo transfer were 36.3% in the ICSI group versus 32.7% in the conventional IVF group (RR 1.11; 95% CI 0.94-1.31; p=0.25). Fertilisation rate per oocyte retrieval and total fertilisation failure rate also did not differ siginficantly between the two groups (55.6% versus 52.7% and 5.5% versus 6.4%, respectively). The mean number of embryos and number of frozen embryos were not significantly different between the two groups, 5.9 ± 4.0 versus 5.7 ± 4.5 and 3.9 ± 2.9 versus 3.8 ± 3.1, respectively. Mean number of embryo transferred were 1.91 ± 0.29 and 1.90 ± 0.30. Implantation rates were 28.5% in the ICSI group versus 28.9% in the conventional IVF group. The rates of clinical pregnancy and miscarriage were 43.0% versus 39.6% (RR 1.08; 95% CI 0.94-1.25; p=0.29) and 4.7% versus 5.1% (RR 0.92; 95% CI 0.54-1.57; p=0.89), respectively.
Limitations, reasons for caution: Double blinding was not possible due to the nature of the interventions. This study excluded couples with a poor fertilisation (≤25%) in previous cycle, which limits its generalizability. The results are based on preliminary analysis, as follow-up of live birth is still ongoing.
Wider implications of the findings: In couples with non-male factor infertility, ICSI did not show significant benefit over conventional IVF. This questions the value of routinely use of ICSI in non-male fertility in routine assisted reproduction.
Trial registration number: NCT03428919

SELECTED ORAL COMMUNICATIONS

SESSION 44: WHAT ARE THE OPTIMAL REGIMES FOR FROZEN EMBRYO TRANSFER?

07 July 2020 Parallel 2 15:15 - 16:30

O-168 Natural cycle frozen-thawed embryo transfer (FET): A comparison of outcomes following blastocyst transfer on day six versus day seven after urinary luteinising hormone (LH) surge.

M. Noble[1], J. Craig[1], A. Bevan[1], T. Child[1,2]

[1]Oxford Fertility, Institute of Reproductive Sciences, Oxford, United Kingdom ; [2]University of Oxford, Department of Women's and Reproductive Health, Oxford, United Kingdom

Study question: In natural cycle FET (NC-FET) is there a difference in clinical outcome between frozen-thawed blastocyst transfer on the sixth compared to the seventh day after urinary luteinising hormone (LH) surge?
Summary answer: We observed double the odds of ongoing pregnancy (≥24 weeks) when frozen-thawed blastocyst transfer occurred on day six rather than day seven after urinary LH-surge
What is known already: Despite the recent rise in the annual number of FET cycles, a large survey of IVF clinics highlighted little consensus between IVF clinics regarding the day of blastocyst transfer in NC-FET. Only two small retrospective studies have compared different days of blastocyst transfer in NC-FET. The first compared blastocyst transfer on day five versus day six after serum LH surge; demonstrating higher live birth rate in the day five group. However, the most recent study demonstrated a similar ongoing pregnancy rate when blastocysts were transferred on day six compared to day seven after a serum LH level of ≥20 IU/L.
Study design, size, duration: We retrospectively analysed all NC-FETs between January 2017 and March 2019, before and after a protocol change (May 2018) from blastocyst transfer occurring on day seven after urinary LH surge (LH+7) to day 6 (LH+6). The primary outcome was **ongoing pregnancy rate ≥24 weeks (OPR)**. We included 558 NC-FET cycles (180 LH+6; 378 LH+7). As an independent control, outcomes from medicated FET were also compared between time periods (651 before, 483 after).
Participants/materials, setting, methods: We included all cycles involving NC-FET of vitrified-thawed, unbiopsied blastocyst(s) derived from autologous oocytes between January 2017 and March 2019 at Oxford Fertility. Outcomes were compared using Chi-squared and multivariate binary logistic regression. The primary outcome was ongoing pregnancy beyond 24 weeks. Over the time period other aspects of clinical practice remained unchanged and, as an independent control, outcomes from medicated FER cycles were also compared between time periods.
Main results and the role of chance: A total of 774 natural cycle FET cycles were started during the study period and after the inclusion criteria were applied a total of 558 cycles were analysed (180 LH+6; 378 LH+7). Baseline characteristics were similar, including age at FET and oocyte recovery, number of oocytes collected in the fresh cycle, fertilisation method (IVF or ICSI), endometrial thickness, day of blastocyst cryopreservation (day five or six), number of embryos transferred and proportion of top-quality embryos.

Ongoing pregnancy rate (OPR) beyond 24 weeks was significantly higher when blastocyst transfer occurred on LH+6 compared to LH+7 (45.0% v 29.1%, p< 0.0001). Adjusting for potential confounders the adjusted odds ratio was 2.13 (95% CI: 1.44-3.14, p<0.0001). Clinical pregnancy rate (52.2% v 32.8%; aOR 2.28, 95% CI 1.56 – 3.31, p<0.0001) and biochemical pregnancy rate (61.7% v 42.1%; aOR 2.26, 95% CI 1.56 – 3.30, p<0.0001) were also significantly (p<0.0001) higher in the LH+6 group. Miscarriage rate was similar between groups (26.1% v 30.2%, aOR 0.75 95% CI 0.42- 1.32, p=0.317).

The OPR for medicated FET was stable between time periods (38.9% after v 43.0% before; aOR 0.90, 95%CI: 0.70-1.16, p=0.41).
Limitations, reasons for caution: Our study is not randomised and compares groups before and after change in practice, rather than patients undergoing treatment contemporaneously. The fact that OPR for our independent control, medicated FET cycles, remained stable between the same time periods is reassuring. However, randomised prospective studies are needed to confirm our findings.
Wider implications of the findings: We observed double the odds of OPR (≥24 weeks) in cycles in which blastocyst transfer occurred at urinary LH+6 compared to LH+7, which is highly significant. Moreover, the high OPR highlights NC-FET with urinary LH testing as an effective, convenient and elegant method of FET in ovulatory women.
Trial registration number: Not applicable

O-169 Pregnancy outcome and cost-effectiveness comparisons of artificial cycle-prepared frozen embryo transfer with or without GnRH agonist pretreatment for polycystic ovary syndrome: a randomized controlled trial

L. Luo[1], Q. Wang[1]

[1]1st affiliated hospital of Sun Yat-Sen University, Center of Reproductive Medicine, Guangzhou, China

Study question: Can gonadotropin releasing hormone agonist (GnRH-a) pretreatment improve pregnancy outcome of artificial cycle-prepared frozen embryo transfer (AC-FET) for women with polycystic ovary syndrome (PCOS)?
Summary answer: For women with PCOS, GnRH-a pretreatment prior to AC-FET does not improve live birth rate but increases direct costs compared to AC-FET without GnRH-a pretreatment.

What is known already: For women with normal ovulation, randomized controlled trials (RTCs) and meta-analyses have reported similar live birth rates after AC-FET with or without GnRH-a pretreatment. However, two recent retrospective studies reported that AC-FET with GnRH-a pretreatment improved pregnancy outcomes for women with PCOS.
Study design, size, duration: A total of 342 women with PCOS (24–40 years of age) scheduled for AC-FET from January 2017 to November 2018 were enrolled in this RCT. Of these patients, 170 received AC-FET without GnRH-a pretreatment (AC group) and 172 with GnRH-a pretreatment (G-AC group). A total of 328 FET cycles were analyzed. Primary outcome was live birth rate and secondary outcomes were clinical pregnancy and early pregnancy loss rates. A cost-efficiency analysis was also performed.
Participants/materials, setting, methods: This study was conducted at the reproductive center of a university-affiliated hospital. Frozen embryos for transfer were obtained from the first ART cycle, and no patient received more than two cycles. All patients included received blastocyst transfer.
Main results and the role of chance: Neither live birth rate per FET cycle nor live birth rate per embryo transferred differed significantly between AC and G-AC groups [92/163 (55.8%) vs. 85/165 (51.5%) and 92/234 (39.3%) vs. 85/245 (34.7%), respectively; both P>0.05]. Clinical pregnancy and early pregnancy loss rates were also comparable between groups. The median direct cost was significantly lower in the AC group compared to the G-AC group (4526.6 RMB vs. 7984.0 RMB, P<0.001). The median direct cost per live birth was also significantly lower in the AC group (8117.6 RMB vs. 15415.1.0 RMB, P<0.001).
Limitations, reasons for caution: Indirect costs during the FET cycle and costs during the whole pregnancy period were not analyzed. Pregnancy-related complications and neonatal outcomes were also not analyzed.
Wider implications of the findings: For women with PCOS, endometrial preparation for FET was equally effective with and without GnRH-a pretreatment, but the direct cost was much higher with GnRH-a pretreatment.
Trial registration number: 81701403

O-170 Safety and IVF outcomes of Day 6 vitrified blastocyst transfers : comparison with Day 5 vitrified blastocyst transfers with propensity score matching

D.S. Park[1], S.W. Lyu[1], H.M. Park[1], J.H. Jeong[1], W.S. Lee[1]

[1]Fertility Center of CHA Gangnam Medical Center, Department of Obstetrics and Gynecology, Seoul, Korea- South

Study question: Does day 6 (D5) vitrified blastocyst transfer (VBT) have similar live birth rate (LBR) with day 5 (D6) VBT?
Summary answer: D6 group has significant lower LBR than D5 group in propensity score matching study.
What is known already: In fresh embryo transfer cycles, D6 blastocyst has significantly lower pregnancy rate compared with D5 blastocyst. However, it is unclear whether this result is maintained in VBT cycles, because of conflicting studies.
Study design, size, duration: This is retrospective cohort study with propensity score matching. We evaluated 1157 cycles of VBT performed between January 2014 and December 2015, at the Fertility Center of CHA Gangnam Medical Center.
Participants/materials, setting, methods: All VBT cycles were allocated to two groups according to the day of blastulation: There were 933 VBT cycles of D5 group and 224 VBT cycles of D6 group. The propensity scores were calculated using binary logistic regression analyses based on the patient and cycle baseline variables. The matched ratio for D5 vs D6 was 3:1, respectively. All patients were underwent natural endometrial preparation.
Main results and the role of chance: After matching, there were no significant differences of baseline characteristics between D5 and D6 groups. D6 group had significantly lower implantation rate (IR), clinical pregnancy rate (CPR) and LBR (IR 44.2% vs. 53.1%, p=0.023; CPR 48.4% vs. 60.4, p=0.009, LBR 33.5% vs. 51.8%, p<0.001). There were no significant differences in the ectopic pregnancy rate (1.3% vs. 2.8%, p=0.458) and multiple pregnancy rate (29.3% vs. 30.2%, p=0.816). Miscarriage rate was higher in Day 6 group (29.3% vs. 10.7%, p<0.001). Neonatal outcomes of D5 and D6 groups were not significantly different.
Limitations, reasons for caution: Although we performed analysis using propensity score matching to control for potential confounders between the study groups, retrospective design itself has limitations.
Wider implications of the findings: If there are no differences with morphological grade between day 5 and day 6 blastocysts, it should be considered to transfer day 5 vitrified blastocysts first in order to shorten time to live birth.
Trial registration number: not applicable

O-171 Comparison of day-5 serum progesterone levels using different routes of administration for artificial endometrial preparation: Intrapatient variation.

M. Cerrillo Martínez[1], G. Nardini[1], M. Cruz[2], M. Mayoral[1], M. Toribio[3], E. Labarta[4], J.A. García Velasco[5]

[1]IVI Madrid, Reproductive Medicine, Madrid, Spain ;
[2]IVI Madrid, embriology, Madrid, Spain ;
[3]IVI Madrid, Nurse department, Madrid, Spain ;
[4]IVI Valencia, Reproductive Medicine, Madrid, Spain ;
[5]IVI Madrid- URJC, Reproductive Medicine, Madrid, Spain

Study question: Is there any variability on the day-5 serum progesterone levels during artificial endometrial preparation using different routes of administration for Luteal phase (LP)?
Summary answer: Day-5 serum progesterone levels seem to be maintained above 9.2 ng/mL irrespective of the route of administration.
What is known already: Progesterone for artificial endometrial preparation can be administered orally, vaginally, subcutaneously or intramuscularly. Although the use of different P4 preparations differ among countries and physicians, vaginal route seems to be the most preferred one, but new options for luteal support are available nowadays. Previous studies suggest that a minimum threshold of day-5 serum progesterone levels needs to be reached during hormonal replacement therapy in order to improve ongoing pregnancy rates. However, there is controversy on the efficacy of distinct types of progesterone preparations and routes of administration. More studies are needed to fully understand the physiology of progesterone in ART
Study design, size, duration: Prospective cross-over pilot study performed between January and June 2019 in IVI-RMA Global clinic, Madrid, Spain. We aimed to evaluate if there is significant intrapatient variation on the day-5 serum progesterone levels with the use of different routes of administration during hormonal replacement therapy. IRB approval 1809-MAD-063-MC
Participants/materials, setting, methods: Three different protocols were administered to 5 participants underwent 3 consequtive cycles of HRT. LP support consisted on 1°) vaginal progesterone 400 mg/12 hours ; 2°) subcutaneous progesterone 25 mg/12 hours ; and 3°) intramuscular progesterone 50 mg / 24 h . After estrogen priming for 10 days, P4 was given for 5 days, with daily serum P were performed. Linear regression model accounted for progesterone levels (response variable) and route of administration (explanatory variable).
Main results and the role of chance: The mean day-5 serum progesterone levels depending on the route of administration were as follows a) 14.6 ± 5.5 ng/mL; b) 47.9 ± 22.3 ng/mL; and c) 60.3 ± 65.5 ng/mL. The linear regression model showed that changing vaginal progesterone to either subcutaneous or intramuscular route had a statistically significant effect on serum progesterone levels, with an average increase of 25 ng/mL (5.4-45.1 ng/mL; p=0.01) using the subcutaneous route and 32 ng/mL (13-52.7 ng/mL; p=0.001) with the intramuscular route. The mixed effects model proved that the subcutaneous preparation maintained the mean progesterone level at 29.5 ng/mL (SE: 7.6, fixed effect), while the intramuscular preparation showed a mean value of 18.1 ng/mL (SE: 9.75, fixed effect). Age and body mass index did not produce a significant effect on the results of the mixed model analysis. Despite the route of administration, all progesterone preparations achieved a mean day-5 serum level above the threshold of 9.2 ng/mL, which is considered the optimal cutoff according to a previous prospective cohort. Of note, one patient did not reach the recommended day-5 concentration with vaginal progesterone.
Limitations, reasons for caution: This is a cross-over pilot study with preliminary data and few patients. Further studies are needed to better understand circulating progesterone levels during hormonal replacement therapy and its impact on reproductive outcomes. Nevertheless, considering the scarcity of data, our results provide important insights and draw attention to intrapatient variability.
Wider implications of the findings: Apparently, the three types of progesterone preparations lead to sufficient circulating hormone levels in the day of

the embryo transfer. However, in the era of personalized reproductive medicine, endometrial preparation and luteal phase support deserve further investigation and patient-specific behaviors must be taken into account.
Trial registration number: NONE

O-172 Frozen-thawed embryo transfer in natural cycles leads to higher clinical pregnancy rates compared to artificial endometrial preparation cycles

N. Aslih[1], Y. Atzmon[1], M. Michaeli[1], A. Bilgory[1], E. Shalom-Paz[1]
[1]*Hillel Yaffe Medical Center, IVF unit, Hadera, Israel*

Study question: Which endometrial preparation protocol in Frozen Embryo Transfer (FET) Cycles offers the optimal results?
Summary answer: Frozen-thawed embryo transfer in natural cycles leads to higher clinical pregnancy rates compared to artificial endometrial preparation cycles.
What is known already: Number of FET Cycles has risen dramatically through the years due to several factors including: efficient cryopreservation strategies (vitrification), elective single embryo transfer strategies which lead to a higher number frozen embryos and transfers per Ovum Pick Up (OPU) and increasing use of Pre Gestational Diagnosis and Screening (PGD/PGS) techniques. The optimal protocol for endometrial preparation has yet to be determined. Artificial preparation of the endometrium is a well-established protocol offering a more flexible time table and patient monitoring. On the other hand, raising experience with natural cycles for FET suggests non-inferior results.
Study design, size, duration: A retrospective study. 703 FET cycles performed in the years 2016-2018 were analyzed. Cycles were divided into two groups: Group A – artificial endometrial preparations (n=390), Group B – natural cycle (NC FET) (n=313).
Participants/materials, setting, methods: Group A – FET with artificial endometrial preparation by exogenous estrogen (Estrofem®, Novo Nordisk) and Progesterone preparations (Endometrin®, Ferring or Duphastone®, Abbott or Crinone®, Merk Serono).Group B – FET based on ovulation cycles included: natural ovulation cycles, modified natural cycles using hCG (Ovitrelle Merck-Serono) for the final triggering of spontaneous and Letrozole induces ovulation.Data collection was performed including demographics, causes infertility, history of fertility treatments, current fresh cycle details and FET cycle outcomes.
Main results and the role of chance: both groups were comparable in terms of patient characteristics, demographics, infertility causes, treatment protocols and number of embryos transferred. Although the mean ESHRE score of the transferred embryos was significantly lower in group B, we found **a** significantly higher clinical pregnancy rate in Group B (NC FET) compared to Group A (Artificial endometrial Preparation), 43.1% Vs. 30.9%, P value = 0.021.
Limitations, reasons for caution: A retrospective study, included different protocols of ovulation cycle for FET.
Wider implications of the findings: large Randomized Controlled Trials are needed to establish the superiority of natural cycle protocol over the artificial endometrial preparation protocols for FET.
Trial registration number: Not Applicable

SELECTED ORAL COMMUNICATIONS

SESSION 45: SPERMATOGENESIS SUBTLE REGULATORY EFFECTORS

07 July 2020 Parallel 3 15:15 - 16:30

O-173 Environmental pollution affects the protamine-histone content in human sperm and changes their properties making able to promote DNA oxidative damage: the contribution of molecular investigations.

M. PISCOPO[1], G. Lettieri[1], F. Febbraio[2], G. D'Agostino[1], E. Mele[1], C. Cardito[1], T. Notari[3], L. Montano[4]
[1]*University of Naples, Biology, Naples, Italy ;*
[2]*Institute of Biochemistry and Cellular Biology, National Research Council CNR, Naples, Italy ;*
[3]*Reproductive Medicine Unit of Check Up Polydiagnostic Center, GEA - Gynecology Embryology Andrology, Salerno, Italy ;*
[4]*Andrology Unit of the "S. Francesco d'Assisi" Hospital, Local Health Authority ASL Salerno, Salerno, Italy*

Study question: Does the content and properties of DNA binding proteins in human sperm change in response to environmental pollution?
Summary answer: We provide evidences that environmental pollution alters human sperm protein framework, their binding to DNA and makes these proteins able to promote DNA oxidative damage.
What is known already: The nucleohistone–nucleoprotamine transition is fundamental in order to ensure condensation of the sperm nucleus into a compact hydrodynamic shape and a protection of the DNA delivered by the spermatozoa. The exposure to many environmental contaminants produce sperm DNA damage. We have already reported a significant increase of sperm telomere length, a higher DNA fragmentation index, and a lower antioxidant activity in males recruited in high environmental impact areas. The major cause of male infertility seems to be sperm DNA damage but the molecular mechanisms which lead to DNA break are not yet fully understood.
Study design, size, duration: In order to investigate the impact of environmental pollution on molecular alterations of human sperm, we collected, sperm samples from 240 healthy males living in high and low environmental impact areas of Campania (Italy). We extracted and characterized the sperm protein content, analyzed their DNA-binding affinity and investigated on the possibility that these proteins participate to oxidative DNA damage. The study was performed from October 2017 to December 2019, within the EcoFoodFertility initiative.
Participants/materials, setting, methods: Sperm samples were obtained with informed consent from 240 healthy males, not presenting other factors affecting semen quality, living in areas at high (n=160) and low (n=80) environmental impact. The sperm proteins were extracted and their electrophoretic pattern characterized. Sperm proteins DNA binding ability was determined by electrophoretic mobility shift assays and fluorescence measurements. The involvement of sperm proteins in DNA oxidative damage was evaluated by their ability to convert supercoiled to relaxed plasmid DNA.
Main results and the role of chance: We found, as expected, a canonical protamines/histones ratio in the 95.06% of people living in low-impact area. Differently, this result was observed only in the 16.61% of samples from males living in high environmental impact areas. In these latter about the 62% showed the presence of only histone, and the remaining 21.78% a not canonical protamines/histones ratio. Moreover, also the samples showing a canonical protamines/histone ratio, presented a different DNA binding mode with respect to those from people living in low-impact area. However, our most relevant result was the finding that, in presence of some pollutants, sperm proteins change their protective ability, participating to DNA oxidative damage. This result was particularly marked for those samples presenting a not canonical protamine/histone ratio. The presence of excess of copper and chromium (two heavy metals involved in Fenton-like reaction) found in the semen of people living in high impact areas, could explain the occurrence of DNA oxidative damage. In fact, we have already reported, in other organism, that the binding of these metals to sperm proteins promote DNA break. The differences observed in our work, that are not due to chance, suggest that environmental pollution has impact, at molecular level, on semen quality.
Limitations, reasons for caution: The altered protamine/histone ratio and the DNA oxidative damage promoted by sperm proteins does not necessarily imply infertility in men living in high environmental impact areas. Limitations are due to the impossibility of carrying out *in vitro* fertilization tests.
Wider implications of the findings: Our molecular approaches could represent additional tools for semen quality evaluation and represent a starting point for develop therapeutic protocols to counteract with these types of sperm alterations. Moreover, our findings give new insights into mechanisms of DNA damage, which is implicated also in several human diseases.
Trial registration number: not applicable

O-174 The super-enhancer-driven long non-coding RNAs as key cell identity genes define the essential core regulatory circuitry in non-obstructive azoospermia through activation of immune signaling pathway.

"Abstract withdrawn by the authors"

O-175 Beta2-adrenergic receptors are mainly expressed in the terminal piece of the human sperm flagellum

J.L. Girela[1], M. Francou[1], A. De Juan[1], J. De Juan[2]

[1]University of Alicante, Biotechnology, San Vicente Del Raspeig, Spain ;
[2]University of Alicante, Instituto Universitario de Estudios de Genero, San Vicente Del Raspeig, Spain

Study question: How is the expresión and distribution of beta2-adrenergic receptors (ADRB2) in human sperm?
Summary answer: Using ultra high resolution microscopy techniques we indentified that the expression of ADRB2 is mainly restricted to the terminal piece of the human sperm flagellum.
What is known already: Recent studies have shown that spermatozoa have very complex machinery for cell signalling. As part of that machinery, numerous neuroreceptors are expressed in the sperm membrane. Human sperm is regionalised, with several physiological processes restricted to determined areas, being therefore expected that specific molecules involved in those processes should also be restricted to that area. Although indirect methods had determined the presence of ADRB2 in human sperm, there is no direct evidence nor the description of the localisation of that receptor. ADRB2 is the direct target of medications used to treat health problems like Hypertension (beta blockers) or asthma (bronchodilators).
Study design, size, duration: Sperm from young healthy donors (n =7) were included in the study. All the samples were normozoospermic according to WHO 2010 criteria. As a positive control, we used the neuroblastoma cell line SH-SY5Y known to express ADRB2. We design the study by developing new techniques that allow us to use high- resolution microscopes (i.e. Laser Scanning Confocal Microscopy LSCM and Field Emission Scanning Electron Microscopy or FE-SEM) to identify the localisation and distribution of ADRB2
Participants/materials, setting, methods: To identify the presence of messenger and protein we used RT-PCR and Western Blot techniques respectively. Immunolabelling techniques were performed by using the same anti-ADRB2 primary antibody followed by the fluorochrome-conjugated secondary antibody for LSCM or gold conjugated for FE-SEM. Negative controls were performed by omitting the primary antibody. In FE-SEM images we quantify the number of gold nanoparticles in the sperm cells and in the background to determine the specificity of the technique.
Main results and the role of chance: We confirm the presence of both messenger and ADRB2 protein in human sperm cells in all the samples, demonstrating the expression of the receptor. LSCM images showed a predominant distribution of the ADRB2 receptor on the Sperm flagellum, with and brighter signal at the terminal piece. In the FE-SEM images, the mean number of gold particles found in the end piece of the sperm flagellum was significantly higher than the one found in the mid and principal piece ($p<0.001$).
Limitations, reasons for caution: The present study only shows the presence of the receptors on the sperm plasma membrane. The activity of those receptors should be investigated by developing bioassays based on the use of agonist and/or antagonist. A more reliable genetic overexpression or knockout approach was not possible in human sperm.
Wider implications of the findings: The presence of ADRB2 in the flagellum of human sperm cells is in concordance with the suggested function of that receptor, the regulation of sperm motility and hyperactivation. It may also suggest a possible side effect of betablockers or bronchodilators with the male fertility potential that needs to be investigated.
Trial registration number: Not applicable

O-176 A novel approach for male contraception: modulation of phosphoprotein phosphatase 1 complexes using bioportides

J. Silva[1], M.J. Freitas[1], J. Santiago[1], S. Jones[2], S. Guimarães[3], S. Vijayaraghavan[4], G. Colombo[5], J. Howl[2], M. Fardilha[1]

[1]iBiMED - Instituto de Biomedicina, Universidade de Aveiro, Aveiro, Portugal ;
[2]Research Institute in Healthcare Science, University of Wolverhampton, Wolverhampton, United Kingdom ;
[3]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal ;
[4]Kent State University, Kent State University, Kent, U.S.A. ;
[5]University of Pavia, Department of Chemistry, Pavia, Italy

Study question: Are bioportides capable of disrupting Phosphoprotein Phosphatase 1 (PPP1) complexes as a strategy to modulate sperm motility?
Summary answer: A cell penetrating peptide (CPP) was able to deliver a peptide as a sychnologically-organized bioportide into spermatozoa with a significant impact on motility.
What is known already: Phosphoprotein phosphatase 1 catalytic subunit gamma 2 (PPP1CC2) is a PPP1 isoform restricted to testicular germ cells and spermatozoa and its inhibition is essential for sperm motility acquisition. PPP1 specificity and activity is regulated by the interaction with PPP1-interacting proteins. The mechanism of sperm motility acquisition is a perfect target for a new male contraceptive since it affects only the post-testicular sperm maturation.
Study design, size, duration: We designed a peptide based on the region including the PPP1 binding motif of a sperm-specific interactor (A-kinase anchor protein 4, AKAP4) covalently coupled to inert CPP (penetratin) as a sychnologically-organized bioportide construct. A mutated homologue was also designed as a control. We evaluated the intracellular accumulation of the bioportide in normozoospermic human sperm and its impact on sperm viability and motility at different time-points.
Participants/materials, setting, methods: Molecular dynamics simulations were used to design the bioportide, that was synthetized by microwave-assisted solid phase. The complex disruption was assessed by blot overlay. Fluorescent cell imaging analysis was used to study the intracellular accumulation of the bioportide in human sperm. Sperm viability was evaluated with the trypan blue test and sperm motility was monitored by computer-assisted sperm analysis.
Main results and the role of chance: In vitro studies revealed that the bioportide was able to disrupt the PPP1CC2/AKAP4 complex. Exposure of human sperm to the bioportide led to a significant decrease in both fast (mean decrease of 83.9 ± 15.8 % compared with negative control; 79.0 ± 24.8 % compared with mutated control; $p<0.01$) and slow progressive motility (mean decrease of 74,1 ± 24,1 % compared with negative control; 72,0 ± 28,5% compared with mutated control; $p<0.01$) and a significant increase in the percentage of immotile sperm (mean increase of 220.4 ± 29.6 % compared with negative control; and 148.2 ± 68.2 % compared with mutated control; $p<0.01$), with no impact on sperm viability. No significant alterations were observed in non-progressive motility. No time-dependent effect was observed.
Limitations, reasons for caution: Studies in animal models are needed to validate these results and investigate pre-clinical safety and feasibility.
Wider implications of the findings: This study exploited two ground-breaking areas of the pharmaceutical field by combining CPP technology with protein-protein interaction modulators to generate a bioportide capable of blocking sperm motility by disrupting a sperm-specific PPP1 complex. This approach has the potential to allow the development of a "male pill" to arrest sperm motility.
Trial registration number: not applicable

O-177 In vitro co-exposure to Benzo(a)pyrene (B(a)P) and aged cerium dioxide nanoparticles (CeO2NPs) enhances DNA damage in human and rat gametes

J. Perrin[1], M. Cotena[2], V. Tassistro[2], C. Sunyach[2], J. Rose[3], M. Auffan[3]

[1]Ap-hm, CECOS Centre clinicobiologique d'AMP CHU La Conception, Marseille Cedex 5, France ;
[2]aix marseille university, Institut Méditerranéen de Biodiversité et d'Ecologie, Marseille, France ;
[3]CNRS, Centre Européen de Recherche et d'Enseignement en Géosciences de l'Environnement, Aix en Provence, France

Study question: Do the addition of Benzo(a)pyrene (B(a)P) enhance the DNA damage induced by *in vitro* exposure to aged cerium nanoparticles (CeO_2NPs) in human and rat gametes?

Summary answer: *In vitro* gametes co-exposure to aged CeO_2NPs + B(a)P induced significant higher DNA damage than exposure to CeO_2NPs alone in human and rat gametes.

What is known already: Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants affecting millions of humans through environmental, dietary and occupational sources. B(a)P is a PAH, known for its carcinogenic and reprotoxic potential. Higher DNA damage was observed in Cumulus - Oocyte Complexes of B(a)P-exposed mice than in those of vehicle controls after oral administration. CeO_2NPs are widely used as diesel additive to increase fuel economy; during combustion, they are associated to PAH and then released in air. Pristine CeO_2NPs affect *in vitro* fertilization in mice and induce significant DNA damage in human sperm. The potential hazard due to the co-exposure CeO_2NPs-B(a)P is still unknown.

Study design, size, duration: Pristine CeO_2NPs were extracted from Envirox™ diesel additive and combusted at 850°C, (average combustion temperature in a diesel engine), to obtain aged CeO_2NPs, which were physico-chemically characterized. B(a)P was purchased from Sigma-Aldrich. Rat gametes were sampled in epididymis and oviducts after cervical dislocation of sexually mature males and 26-days-old females (after ovarian stimulation). Human frozen sperm from fertile donors were purchased from Germetheque biobank (France).

Participants/materials, setting, methods: Human and rat gametes were exposed *in vitro* to very low concentrations of CeO_2NPs (1 $\mu g.l^{-1}$), to B(a)P alone (1.13µM) and to 1 $\mu g.l^{-1}$ CeO_2NPs + 1.13µM B(a)P during 1 hour in Ferticult© Medium + 1% DMSO + 1% S9mix at 37°C, 5% CO_2. DNA damage was analysed by alkaline comet assay (ACA) and quantified by Olive Tail Moment (OTM) in oocytes and follicle cells and by % Tail DNA in sperm.

Main results and the role of chance: In human sperm, co-exposure to CeO_2NPs + B(a)P induced significantly higher DNA damage (mean % Tail DNA±SEM = 35.35 ± 0.75) compared to CeO_2NPs alone (29.57 ± 0.68) ($p < 0.0001$) and B(a)P alone (18.07 ± 0.79), ($p < 0.0001$).

In rat sperm, co-exposure induced significantly higher DNA damage (mean %Tail DNA±SEM = 32.4 ± 0.43) compared to CeO_2NPs alone (22.73 ± 0.43) ($p < 0.0001$) and B(a)P alone (13.91 ± 0.44), ($p < 0.0001$).

In rat follicle cells, co-exposure induced significantly higher DNA damage (mean±SEM OTM = 8.48 ±0.35) compared to CeO_2NPs alone (4.31 ± 0.30) ($p < 0.0001$) and B(a)P alone (1.83 ± 0.34), ($p < 0.0001$).

In rat oocytes, co-exposure (7.5±0.7) induced a significant increase of DNA damage compared to B(a)P alone (4.28±0.7) ($p = 0.0046$), but not if compared to CeO_2NPs alone (8.43±0.8) ($p >0.05$).

The significant increase in genotoxicity induced by co-exposure to CeO_2NPs and B(a)P suggest the implication of the so-called "Trojan Horse" mechanism by which nanoparticles can act as a carrier of another chemical compound by promoting the interaction with cells. This hypothesis is supported by the results obtained in oocytes, which show a potentialized effect of B(a)P due to the co-exposure with CeO_2NPs.

Limitations, reasons for caution: These results cannot be extrapolated to *in vivo* toxicity of CeO_2NPs + B(a)P after inhalation, but demonstrate that interactions between the mixture CeO_2NPs + B(a)P can significantly and synergistically enhance the DNA damage in human and rat sperm and in rat follicle cells and oocytes.

Wider implications of the findings: These results show for the first time that interactions between aged CeO_2NPs and B(a)P synergistically potentiated toxicological effects on human and rat gametes. This information should help further our understanding of the combined toxicity of PAH and nanoparticles, as PAH are the most ubiquitous organic compound pollutants in ambient air.

Trial registration number: non applicable

SELECTED ORAL COMMUNICATIONS
SESSION 46: REPRODUCTIVE (EPI)GENETICS 2
07 July 2020 Parallel 4 15:15 - 16:30

O-178 Non-invasive metabolic imaging with fluorescence lifetime imaging microscopy (FLIM) detects differences in ploidy of human blastocysts

J. Shah[1,2], M. Venturas[3,4], T. Sanchez[5], A. Penzias[1,2], D. Needleman[3], D. Sakkas[2]

[1]*Beth Israel Deaconess Medical Center, Obstetrics and Gynecology, Boston, U.S.A. ;* [2]*Boston IVF, Reproductive Endocrinology and Infertility, Boston, U.S.A. ;* [3]*Harvard University, Molecular and Cellular Biology and School of Engineering and Applied Sciences, Cambridge, U.S.A. ;* [4]*Universitat Autònoma de Barcelona, Department de Biologia Cellular- Fisiologia i Immunologia, Cerdanyola, Spain ;* [5]*None, None, Boston, U.S.A.*

Study question: Can non-invasive metabolic imaging via fluorescence lifetime imaging microscopy (FLIM) detect differences between euploid and aneuploid discarded human blastocysts?

Summary answer: Non-invasive metabolic imaging via FLIM can detect significant differences between embryos deemed aneuploid, euploid and of unknown ploidy status.

What is known already: FLIM of endogenous nicotinamide adenine dinucleotide dehydrogenase (NADH) and flavin adenine dinucleotide (FAD) autofluorescence reveals the metabolic state of mouse embryos. In mouse and other animal models, the metabolic state of embryos correlates with embryo viability. Ploidy status is one of the primary determinants of embryo viability. Metabolic state correlates with ploidy status in a number of non-embryonic systems, including yeast and cancer cells.

Study design, size, duration: This was a prospective observational study that included 158 discarded human vitrified blastocysts of which 110 had known ploidy status (12 euploid; 98 aneuploid) by preimplantation genetic testing and the remaining 48 embryos were of unknown ploidy status. All blastocysts were day 5 or 6 with Gardner morphology grade A or B except 4 that were grade C. Multilevel models were used for analysis with $p<0.05$ showing significance.

Participants/materials, setting, methods: Metabolic imaging via FLIM of NADH and FAD was used in a University affiliated private IVF laboratory to study discarded human blastocysts. Eight metabolic parameters were obtained from each blastocyst (4 for NADH and 4 for FAD): short (t1) and long (t2) fluorescence lifetime, fluorescence intensity (I), and fraction of the molecule engaged with enzyme (F). The redox ratio (Intensity of NADH)/(Intensity of FAD) was also calculated for each embryo.

Main results and the role of chance: Each blastocyst was warmed, incubated for 2 hours and imaged to analyze its metabolic signature. The mean ages in years for the unknown ploidy, euploid, and aneuploid groups were 32.0 ± 4.9, 37.0 ± 4.6, and 36.9 ± 3.7, respectively. In comparing blastocysts from the aneuploid, euploid, and unknown groups, the FLIM parameters of NADH-I ($p<0.0001$), NADH-t2 ($p<0.02$), FAD-I ($p<0.0001$), FAD-t1 ($p<0.001$), and redox ratio ($p<0.0001$) showed significant metabolic differences. When comparing ploidy status to embryo day, a number of significant metabolic differences were seen in the FAD parameters. FAD-F, FAD-t1 and FAD-t2 all showed significant differences ($p<0.01$) when comparing euploid day 5 versus day 6, aneuploid day 5 versus day 6, and unknown day 5 versus day 6. Furthermore, euploid day 5 versus day 6 showed significant differences in redox ratio ($p<0.0001$) while unknown ploidy day 5 versus day 6 showed significant differences in redox ratio and FAD-I ($p<0.02$).

Limitations, reasons for caution: The study was performed using discarded frozen human blastocysts, which may differ metabolically from non-discarded human embryos. Only a small number of blastocysts deemed euploid (12) were assessed. Additional data is required to validate the results.

Wider implications of the findings: FLIM has revealed significant metabolic differences between discarded human blastocysts associated with ploidy status. It is unclear if these associations are correlative or causative (and, if so, if ploidy impacts metabolism or if metabolism impacts ploidy). Further studies are needed to determine if FLIM can assist in clinical embryo selection.

Trial registration number: not applicable

O-179 Clinical outcomes after transfer of 110 euploid and 11 mosaic blastocysts identified using non-invasive preimplantation genetic testing for aneuploidy

J. Zhang[1], M. Olcha[1], M. Elzaky[1], M. Jaremko[1], A. Chavez[2]

[1]*New Hope Fertility Center, Reproductive Endocrinology, New York- NY, U.S.A. ;* [2]*New Hope Fertility Center, Reproductive Endocrinology, Mexico City, Mexico*

Study question: What is the biochemical pregnancy rate after single embryo transfer (SET) of euploid and mosaic embryos identified via non-invasive pre-implantation genetic testing for aneuploidy (PGT-A)
Summary answer: Transfer of 110 euploid and 11 mosaic embryos resulted in a 63.6% and 36.3% positive beta-human chorionic gonadotropin level (ß-hCG), respectively
What is known already: PGT-A has been widely used around the world to identify ploidy status of embryos. The knowledge obtained can help assist clinicians and patients with prioritization of embryos for transfer. The transfer of single euploid embryos has be shown to improve per cycle pregnancy rate, reduce miscarriage rate, and prevent transfer of embryos harboring certain genetic anomalies such as Down Syndrome. The primary modality for obtaining DNA from blastocysts has been trophectoderm biopsy, which requires removal of embryonic cells. Recently, the advent of non-invasive methods have been developed which allow embryonic DNA to be amplified by analysis of culture media only.
Study design, size, duration: Retrospective data analysis of biochemical pregnancy rates after 110 euploid and 11 mosaic embryos were transferred between August 1st 2019 to December 30th 2019.
Participants/materials, setting, methods: Ploidy information on 24 chromosomes was derived by testing spent media collected on Day 5, 6, or 7. Media was processed on site using Non-Invasive Chromosomal Screening (NICS) platform (Yikon Genomics), utilizing multiple annealing and looping-based amplification cycles (MALBAC) for whole-genome amplification followed by next-generation sequencing. Initial ß-hCG levels were analyzed 7-10 days after SET. ß-hCG levels were measured with an automated immunoassay analyzer (TOSOH). Chi squared analysis was used to compare biochemical pregnancy rates.
Main results and the role of chance: A total of 70 out of 110 (63.6%) euploid SETs and 4 out of 11 (36.3%) mosaic SETs resulted in a positive ß-hCG. As expected, the pregnancy rate after euploid embryo transfer was significantly higher than mosaic embryo transfer (p=0.03).
Limitations, reasons for caution: A small study size is the major limitation. Additionally, caution should be taken when generalizing results as there are many other clinical and embryologic practice parameters that could affect pregnancy rates in general.
Wider implications of the findings: This data helps demonstrate that a non-invasive PGT-A platform can be utilized successfully in a private setting without compromising clinical outcomes. Furthermore, the use of a non-invasive methods to identify embryo ploidy may reduce risk to the embryo, however this has yet to be determined
Trial registration number: not applicable

O-180 Single tube long fragment read (stLFR) with massively parallel sequencing achieved patient-only haplotyping for preimplantation genetic testing of monogenic disorders

S. Chen[1], X. Cheng[2], D. Chen[2], H. Yan[2], J. Xia[2], P. Liu[2], X. Yin[2], F. Chen[2], C. Xu[1]

[1]The International Peace Maternity and Child Health Hospital- School of Medicine- Shanghai Jiao Tong University, Reproductive Genetics Department, Shanghai, China ;
[2]MGI- BGI-Shenzhen, R&D center, Shenzhen, China

Study question: Can massively parallel sequencing (MPS) offer an approach for preimplantation genetic testing of monogenic disorders (PGT-M) haplotyping with only a patient?
Summary answer: Single tube long fragment read (stLFR) with MPS achieved patient-only haplotyping for PGT-M.
What is known already: Several MPS-based PGT methods have been developed including low-depth MPS such as OnePGT, MARSALA and haploseek as well as high-depth MPS. Parents-child trios or parents with additional family members are required for PGT-M haplotyping with low-depth MPS. Moreover, a reference embryo can be used for phasing, that parents-only haplotyping without proband can be achieved by relative high-depth MPS. PGT-M haplotyping for parents-only has been reported to be performed by stLFR previously. However, PGT-M haplotyping for patient-only in some cases that the spouse is uncertain or unavailable have not been reported.
Study design, size, duration: We recruited 2 families that had undergone *in vitro*fertilization (IVF) and traditional PGT-M treatment. Each participant was on an informed-choice basis and signed a written consent. Both families were with parental-proband trios and the offspring monogenic disorder was inherited from only one spouse (both the wives). We obtained 12 and 5 embryos for the 2 families, respectively. Parental-proband trio haplotyping and PCR-Sanger sequencing would validate the result of patient-only haplotyping.
Participants/materials, setting, methods: Embryos were trophectoderm biopsied in Day5 blastocyst stage, and used for Qiagen REPLI-g Kit whole genome amplification (WGA). High molecular weight genomic DNA was extracted from the spouse patient peripheral blood, and used for MGIEasy stLFR Library Prep Kit (Item No.1000005622) according to instructions. Regular WGS libraries from WGA DNA and family members bulk DNA, together with spouse patient stLFR libraries were used for DNBSEQ-G400 sequencing with 15X depth each sample and haplotype analysis.
Main results and the role of chance: According to the co-barcoding information in the spouse patient(both the wives) stLFR sequencing data, we phased the pathogenic mutationCOL4A5 c.1834G>C and COL1A1 c.769G>A in the 2 families, and confirmed the pathogenic maternal haplotype linkage with the mutation allele by patient-only haplotyping. Combining the pathogenic haplotype information and embryonic informative SNP alleles, we determined the genetic status of monogenic disorders for each embryo. In COL4A5 family, 7 embryos were affected with pathogenic maternal haplotype and 5 embryos were normal. In COL1A1 family, 2 embryos were affected and 3 embryos were normal. The patient-only haplotyping results were validated by parental-proband trio haplotyping and were 100% concordant. PCR-Sanger sequencing of mutationCOL4A5 c.1834G>C and COL1A1 c.769G>A in the 2 families, respectively, showed 100% concordance with the patient-only haplotyping. PGT for aneuploidy (PGT-A) was performed by MPS. In COL4A5 family, 2 aneuploidy embryos and 10 euploidy embryos were tested. In COL1A1 family, 3 aneuploidy embryos and 2 euploidy embryos were determined. The MPS PGT-A results were validated by SNP-array and showed 100% concordant. Three embryos inCOL4A5 family and one embryo in COL1A1 family were both euploidy and with normal maternal haplotype that were available for implantation.
Limitations, reasons for caution: The patient-only PGT-M haplotyping by stLFR MPS is applicable in the families that only one spouse carry the pathogenic mutation. Otherwise, parents-only PGT-M haplotyping by stLFR MPS can be performed.
Wider implications of the findings: The patient-only PGT-M haplotyping by stLFR MPS provides an effective PGT solution for patients with IVF artificial insemination by donor (AID) or patients with unavailable spouse. Both patient-only and parents-only haplotyping provide complement to the current PGT methodology and can be clinically applied according to the actual situations.
Trial registration number: Not applicable

O-181 First report about preimplantation genetic testing for the Xq24 microdeletion related to X-linked mental retardation, Nascimento type in a woman with recurrent pregnancy loss

I. Lebedev[1], E. Soloveva[1], D. Zhigalina[1], L. Minaycheva[1], O. Kanbekova[1,2], A. Nemtsova[2], K. Tolmacheva[1], E. Fonova[1], N. Skryabin[1], A. Kashevarova[1], M. Lopatkina[1], I. Stepanov[2], L. Nazarenko[1], A. Svetlakov[1]

[1]Research Institute of Medical Genetics, Tomsk National Research Medical Center, Tomsk, Russia C.I.S. ;
[2]Tomsk Regional Perinatal Center named after I.D. Evtushenko, ART Department, Tomsk, Russia C.I.S.

Study question: Can the presence of inherited chromosomal microdeletions associated with microdeletion syndromes be effectively detected in human preimplantation embryos by current PGT approaches?
Summary answer: Combining STR-haplotyping (PGT-M) and array-CGH (PGT-A) provides high-resolution detection of inherited chromosomal microdeletions less than 300 kb in size in human preimplantation embryos.
What is known already: Copy number variations (CNVs), including chromosomal microdeletions and microduplications less than 5 Mb in size, have significant impacts on human pathology as they are strongly associated with intellectual disability, developmental delay and congenital malformations. The average resolution of current PGT-A and PGT-SR technologies, including NGS and array-CGH, is approximately 5-10 Mb, ruling out the possibility of preimplantation genetic testing for the most severe microdeletion and microduplication syndromes. Moreover,

segmental aneuploidies lack any genotype-phenotype correlations at the blastocyst stage. However, some CNVs have low penetrance and can be inherited from healthy parents, providing a chance to be detected in preimplantation embryos.
Study design, size, duration: Here, we report about preimplantation genetic testing of a 270-kb Xq24 microdeletion associated with X-linked syndromic mental retardation, Nascimento type (OMIM 300860) by combining STR-haplotyping and array-CGH in a couple with recurrent pregnancy loss. A previously investigated woman and her mother are carriers of the Xq24 microdeletion, as detected by array-CGH and confirmed by quantitative real-time PCR, but did not demonstrate the classical syndromic phenotype due to extremely skewed X-chromosome inactivation (sXCI).
Participants/materials, setting, methods: A 30-year-old woman had 8 pregnancies; one was terminated by a medical abortion; six were spontaneous abortions, including two males with a Xq24 microdeletion and one female without a microdeletion but random XCI. The woman also gave birth to a healthy girl without microdeletion and random XCI. PGT was performed using WGA (REPLI-g MDA, Qiagen), nested-PCR for STR markers within deleted, 0.8 Mb upstream and 1.0 downstream regions and array-CGH (GenetiSure Pre-Screen Microarray, Agilent Technologies).
Main results and the role of chance: To develop a system for preimplantation STR-haplotyping PGT setup was performed as for monogenic disorders. Family DNA samples were used to haplotype normal and mutant X-chromosomes. Seventeen polymorphic STR markers were selected and tested in the PGT-M setup, and 12 were approved as informative. The system was validated in single cells. Nine oocytes were fertilized by ICSI, and 8 zygotes were obtained in the IVF programme with standard ovarian stimulation. Trophectoderm biopsy was performed for 4 blastocysts on Days 5-6 followed by WGA by MDA, which was successful for 3 samples. STR-haplotyping combined with testing for *AMEL* and *SRY* genes revealed a hemizygous Xq24 microdeletion in male embryo, heterozygous carrier female embryo and normal female embryo. Array-CGH revealed monosomy 4 in female carrier embryo sample. Standard array-CGH data processing did not find microdeletion but detailed analysis allowed us to obtain concordant results with regard to the Xq24 microdeletion despite small size rearrangement. The same microdeletion arr[hg19] Xq24(118518668_118836030) like in the patient was also detected in hemizygous male and heterozygous female carrier embryos. Normal female embryo was recommended for transfer.
Limitations, reasons for caution: Only inherited chromosomal microdeletions can be detected by combining PGT-M and PGT-A. *De novo* microdeletions and microduplications are still missed using PGT approaches. STR-haplotyping requires a preliminary step for selecting heterozygous markers. Risks of heterozygous for X-chromosome microdeletions female embryos transfer are unknown and should be further estimated.
Wider implications of the findings: Our data indicate the successful detection of clinically significant chromosomal microdeletion by combining PGT-M and PGT-A. Implementation of PGT-A reduces the risk of aneuploid embryo transfer. The presented approach provides new strategies for PGT in women with recurrent pregnancy loss and sXCI. This study was supported by RFBR (18-015-00437a).
Trial registration number: not applicable

O-182 Cell-based non-invasive prenatal testing (cbNIPT): an alternative to chorionic villus sampling to confirm diagnosis of unaffected fetuses following preimplantation genetic testing for monogenic disorders (PGT-M)

C.L.F. Toft[1,2], H.J. Ingerslev[3], U.S. Kesmodel[2,3], L. Hatt[4], R. Singh[4], K. Ravn[4], B. Hestbek[4], I.B. Christensen[4], M. Koelvraa[4], L.D. Jeppesen[4], P. Schelde[4], I. Vogel[5], N. Uldbjerg[6], R. Farlie[7], S. Sommer[8], M.L.V. Østergaard[9], A.N. Jensen[10], H. Mogensen[11], B. Degn[1], H. Okkels[1], A. Ernst[1], I.S. Pedersen[1,2]

[1]Department of Molecular Diagnostics, Aalborg University Hospital, Denmark ;
[2]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark ;
[3]Fertility Unit, Aalborg University Hospital, Denmark ;
[4]ARCEDI Biotech ApS, , ;
[5]Clinical Genetic Department, Aarhus University Hospital, Aarhus, Denmark ;
[6]Department of Gynecology and Obstetrics, Aarhus University Hospital, Aarhus, Denmark ;
[7]Department of Obstetrics and Gynecology, Viborg Regional Hospital, Viborg, Denmark ;
[8]Department of Gynecology and Obstetrics, Horsens Regional Hospital, Horsens, Denmark ;
[9]Department of Gynecology and Obstetrics, Randers Regional Hospital, Randers, Denmark ;
[10]Department of Gynecology and Obstetrics, Aalborg University Hospital, Aalborg, Denmark ;
[11]Department of Gynecology and Obstetrics, Kolding Regional Hospital, Kolding, Denmark

Study question: To investigate cell-based non-invasive prenatal testing (cbNIPT) following preimplantation genetic testing for monogenic disorders (PGT-M) as an alternative to invaisve chorionic villus sampling (CVS).
Summary answer: CbNIPT had a high specificity of fetal cell isolation (95 %). Successful diagnosis in agreement with PGT-M and CVS was achieved in all seven cases.
What is known already: Although the risk of misdiagnosis associated with preimplantation genetic testing (PGT) is low (< 1 %), the consequences are severe. Hence, prenatal analysis is recommended folllowing PGT, but only approximately 50 % of patients opt for the procedure. The current golden standard for prenatal analysis is chorionic villous sampling, which is assocated with discomfort and a small risk of miscarriage (< 1 %). It is possible that a more comfortable and non-invasive alternative would increase the proportion of patients receiving prenatal analysis were recommended, thereby increasing the chance of detecting the rare case of misdiagnosis.
Study design, size, duration: Desciptive case-series. Seven patients achieving pregnancy following PGT-M had a blood sample taken on the day of CVS, from which potential fetal cells were isloated using a subset of fetal specific cell surface markers within 48 hours. Fetal cells were analyzed by STR marker analysis and direct mutation detection and compared to the original PGT-M analysis of the transferred embryo and the CVS result. Patients were enrolled between November 2018 and August 2019.
Participants/materials, setting, methods: The following was evaluated for the isolated potential fetal cells:- Number of potential fetal cells isolated in total, the range and median per case - Fraction of cells receiving a diagnosis including range and median per case - Fraction of successfully diagnosed cells of fetal origin including range and median per case - Fraction of fetal cells receiving a diagnosis including range and median per case - Agreement between fetal cells, original PGT-M analysis and CVS analysis.
Main results and the role of chance:

	N (%)	Range	Median per case
Fetal cells isolated	29		5
Fraction of cells receiving a diagnosis	21/29 (72 %)	1-6	2
Fraction of successfully diagnosed cells being of fetal origin	20/21 (95 %)	1-6	2
Fraction of fetal cells receiving a diagnosis	20/20	1-6	2
Agreement between fetal cells, original PGT-M analysis and CVS analysis per case basis.	7/7	-	-

Limitations, reasons for caution: Measures are warranted in order to increase the diagnostic success rate per cell sample, and larger series are needed to validate robustness before cbNIPT can replace CVS in the clinical practice.
Wider implications of the findings: Since cbNIPT can be performed in early pregnancy, as early as gestational week four, results can be obtained in time for CVS to be performed in case of a failed analysis. Hence, cbNIPT can be offered as a primary non-invasive option of prenatal testing.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 47: DOES EMOTIONAL BALANCE BEFORE BEING PARENTS AND AFTER EXIST?

07 July 2020 Parallel 5 15:15 - 16:35

O-183 In-depth analysis of what influences whether patients' commit to achieve parenthood (parenthood-engagement) and undergo fertility treatment (treatment-engagement) before and after a treatment cycle.

S. Gameiro[1], S. Mesquita da Silva[2], U. Gordon[3], G. Baccino[4], J. Boivin[5]

[1]School of Psychology, United Kingdom, Cardiff, United Kingdom ;
[2]University of Oporto, Faculty of Sports, Oporto, Portugal ;
[3]Bristol Fertility Clinic, Bristol Fertility Clinic, Bristol, United Kingdom ;
[4]fivmadrid, fivmadrid, Madrid, Spain ;
[5]Cardiff University, School of Psychology, Cardiff, United Kingdom

Study question: Which factors are associated with patients' commitment/effort to achieve parenthood (parenthood-engagement) and undergo fertility treatment (treatment-engagement) before and after a treatment cycle?
Summary answer: Before the cycle parenthood-engagement and treatment-engagement are associated with stronger chidlwish, after the cycle parenthood-engagement is associated with stronger childwish and treatment-engagement with achieving pregnancy.
What is known already: Infertile patients have to actively engage with their parenthood goal (parenthood-engagement) if they want to achieve it, which often means engaging with fertility treatment (treatment-engagement). Engagement is the level of commitment/effort one puts towards achieving a goal, as opposed to stop pursuing it (disengagement) and pursuing other goals (reengagement). Psychological theories predict that engaging is adaptive (i.e., results in better mental-health) while goals are achievable, but when they become totally blocked it is adaptive to disengage and reengage. Infertility research has provided weak support to these predictions, maybe because of methodological limitations (e.g., poor blockage operationalization, neglect of other predictors).
Study design, size, duration: Prospective study with two moments: before a treatment cycle (T1), 6 weeks after its outcome (T2). Determinants of engagement were participants' strength of childwish, objective (prognosis, cycle outcome) and subjective (perceptions of) blockage, and patient's general ability to disengage and reengage when facing blocked goals. Engagement was expected to mediate associations between determinants and mental-health (anxiety, depression) and was conceptualized in terms of effort/commitment to achieve parenthood (parenthood-engagement) and to continuing with fertility treatment (treatment-engagement).
Participants/materials, setting, methods: The NHS-UK provided ethical approval. Patients starting an IVF, ICSI, IUI cycle using own or donated gametes were consecutively recruited and completed T1 and T2 questionnaires. Childish and subjective blockage were assessed with single-item questions. Objective blockage was prognosis data (T1) and cycle outcome (T2), collected from medical records. General ability to disengage and reengage were measured with the Goal Disengagement and Reengagement Scale, parenthood-engagement with the OPS-scale, treatment-engagement with the FertiQol-persistence, mental-health with HADS.
Main results and the role of chance: The final sample comprised 117 participants (33% response rate, 47% attrition rate). Average age was 35.81 (SD=4.14) years, 77 (66%) were women, 19 (16%) had children and 49 (42%) achieved pregnancy on their treatment cycle.

Mixed ANOVAs WS:Time(T1, T2) x BS:Outcome(not pregnant, pregnant) were run to investigate changes from T1 to T2 on determinants, engagement and mental-health. Subjective blockage decreased for pregnant participants ($\eta2$=.223,p<.01) but remained stable for non-pregnant ($\eta2$=.036,p >.05). Disengagement ability increased for all participants ($\eta2$=.045,p<.05). Parenthood-engagement decreased ($\eta2$=.104,p<.001) for all participants. Treatment-engagement decreased for non-pregnant participants ($\eta2$=.386,p<.001) but remained stable for pregnant ($\eta2$=.010,p>.05).

Results from mediation analysis (MEDIATE macro; Preacher & Hayes, 2004) showed that, at T1, higher childwish (ß=.40,SE=.09) and lower ability to disengage (ß=-.28,SE=.10) were associated with higher parenthood-engagement. At T2, higher T1 parenhood-engagement (ß=.59,SE=.08) and childwish (ß=.14,SE=.07) and lower subjective blockage (ß=-.14,SE=.07) and ability to disengage (ß=-.18,SE=.07) were associated with higher parenthood-engagement. There were significant associations between parenthood-engagement and mental-health.

At T1, childwish (ß=.35,SE=.08) was associated with higher treatment-engagement and this with lower anxiety (ß=-.33,SE=.12) and depression (ß=-.51,SE=.11). At T2, T1 treatment-engagement (ß=.46,SE=.10), being pregnant (ß=.58,SE=.16) and lower ability to reengage (ß=-.21,SE=.08) were associated with higher treatment-engagement, and this with lower depression (ß=-.25,SE=.09).
Limitations, reasons for caution: Response and attrition rates were similar to other questionnaire studies but worse-prognosis patients were more likely to attrite from the study. Engagement measures were self-reported but their predictive power of undergoing another cycle (n=76 patients without pregnancy/childbirth) was as expected: parenthood-engagement did not predict uptake, OR=.925,95%CI[.811,1.053], but treatment-engagement did, OR=1.261,95%CI[1.060,1.500].
Wider implications of the findings: The forces associated with parenthood and treatment engagement change from before to after a treatment cycle. Before, patients' engagement with parenthood and treatment reflect their strong desire for children. After, they reflect a conflict between patients' desire for children and their immediate emotional reaction to the cycle outcome.
Trial registration number: not-applicable

O-184 Meditation and mindfulness reduce stress in women with recurrent pregnancy loss: A randomized controlled trial

K. Kirchheiner Jensen[1], M.C. Krog[2], A.M. Kolte[2], S. Hedegaard[2], M. Chonovitsch[1], A.L. Lunøe[2], E.C. Koert[2], L. Schmidt[3], H. Svarre Nielsen[1]

[1]Amager Hvidovre University Hospital, Department of Obstetrics and Gynecology, Hvidovre, Denmark ;
[2]Rigshospitalet Copenhagen University Hospital, Recurrent Pregnancy Loss Unit-Fertility Clinic 4071, Copenhagen, Denmark ;
[3]University of Copenhagen, Department of Public Health, Copenhagen, Denmark

Study question: Does a 7-week meditation and mindfulness program reduce perceived stress among women with recurrent pregnancy loss (RPL) ?
Summary answer: A 7-week meditation and mindfulness program significantly reduced perceived stress in women with RPL compared to a standard supportive care program for women with RPL.
What is known already: Previous studies have shown that both perceived stress and moderate-severe depression is significantly more prevalent among women with RPL compared to other women trying to conceive. Despite the severe mental impact on women experiencing RPL, evidence-based care for the psychological consequences is currently limited. Meditation and mindfulness interventions have shown to be beneficial in reducing stress and negative feelings as well as improving well-being for patients with a wide range of medical conditions. However, to our knowledge, it has never been investigated if meditation and mindfulness can reduce stress among women with RPL.
Study design, size, duration: A two-armed randomized controlled trial (RCT) designed to evaluate a meditation and mindfulness intervention for women with RPL versus standard supportive care for women with RPL. Participants were included from November 2018 to April 2019. Of 163 invited, 76 were enrolled and randomly assigned to either supportive care (control group 38 participants) or to a 7-week meditation and mindfulness program lead by a professional instructor in addition to supportive care (intervention group 38 participants).
Participants/materials, setting, methods: Patients in a tertiary RPL unit were invited to participate. The intervention group had three meditation and mindfulness workshops with two to three-week intervals. Additionally, the intervention group meditated daily, supported by an online audio guide. The control group was instructed not to meditate during the 7-week study period. Participants' mental health was evaluated twice by the perceived stress scale,

the major depression inventory, COMPI fertility problem stress scales and COMPI marital benefit measure.
Main results and the role of chance: There were no differences between the groups at baseline. The main outcome, perceived stress, decreased significantly both in the intervention group (mean difference 5, standard deviation 4, p-value 0.001) and in the control group (mean difference 2, standard deviation 4, p-value 0.006). However, the perceived stress score in the intervention group decreased significantly more compared with the control group, p-value 0.027. The infertility-specific stress in the personal domain (COMPI Fertility Problem Stress Scales) decreased significantly in the intervention group (p-value 0.04), otherwise all other secondary outcomes showed no significant differences between the two groups. Seven women left the intervention group after the first session, indicating that it was too stressful to participate, whereas two women left the control group.
Limitations, reasons for caution: It is unknown if participants in the control group meditated, despite being instructed not to. Likewise, we are unaware if the participants in the intervention group meditated as instructed. Meditation and mindfulness can be an overload for women experiencing RPL since seven participants left the intervention group.
Wider implications of the findings: This is the first RCT study investigating the effect of meditation and mindfulness on the mental health of women experiencing RPL. Meditation and mindfulness reduced perceived stress significantly more than our routine supportive care. Guided self-administered meditations could be a useful tool in the care for women experiencing RPL.
Trial registration number: NCT03905395

O-185 The realism of men and women's expected IVF live birth rates

K. Peeraer[1], J. Devroe[1], T. D'Hooghe[2], J. Boivin[3], J. Vriens[4], E. Dancet[1]

[1]Leuven University Hospital, Departement of Development and Regeneration, Leuven, Belgium ;
[2]KU Leuven, Departement of Development and Regeneration, Leuven, Belgium ;
[3]Cardiff University, School of Psychology, Cardiff, United Kingdom ;
[4]Laboratory of Endometrium- Endometriosis & Reproductive Medicine, Department of Development and Regeneration, Leuven, Belgium

Study question: Which live birth rates (LBR) do IVF-patients expect and are they in line with their IVF-prognosis and affected by their gender and general optimism?
Summary answer: During IVF women and especially men expect unrealistically high IVF-LBRs and the difference between men and women is not explained by general level of optimism.
What is known already: The general public is known to overestimate in vitro fertilisation (IVF) success rates. Qualitative interviews showed that well informed women cryopreserving their oocytes were unrealistically optimistic about their chances on a live birth as they thought they and/or their gynaecologist would perform better than average (de Groot et al., 2016). To the best of our knowledge, the LBRs expected by patients during their IVF-cycle have yet to be studied and compared to patient's personalized IVF-prognosis. In addition, whether these expected LBRs are affected by factors like gender and level of optimism is unknown.
Study design, size, duration: We prospectively surveyed the expected LBRs and degree of general optimism of 69 couples having an oocyte aspiration in our clinic between March and December 2019. Men and women were asked to each fill out their own questionnaire. Additionally, couples' personalized IVF-prognosis was calculated using the van Loendersloot prognostic model (van Loendersloot et al., 2013, Sarais et al.,2016) after validation and calibration on our clinics data (c-statistic of 0.74).
Participants/materials, setting, methods: Eligible couples completed at least one 2nd-6th IVF-cycle with own gametes after a previous IVF-cycle with the same partner in our clinic. The level of general optimism was assessed with the reliable 'LOT-R' questionnaire (Scheier et al., 1994). The degree of mis-estimation was calculated with the following formula: (expected IVF-LBR - prognosis)/prognosis. A positive sign shows overestimation, a negative sign shows underestimation and the absolute value quantifies the extend of mis-estimation.
Main results and the role of chance: The 67 participating couples (participation rate=97.10%) had a mean IVF-prognosis (i.e. calculated LBR per completed IVF-cycle, including fresh and frozen embryo transfers) of 31.8% (range=4.8%-59.4%; SD=16.9). Eighty-five percent of women over-estimated their IVF-LBR (mean overestimation 33.66%; SD=20.02) and 47.8% even expected their IVF-LBR to be more than double their calculated IVF-prognosis (mean overestimation 46.47%; SD=16.10). Eighty-eight percent of men over-estimated their IVF-LBR (mean overestimation 38.81, SD=21.84) and 53.7% even expected their IVF-LBR to be more than double their calculated IVF-prognosis (mean overestimation 51.10%; SD=17.75). Men expected significantly higher IVF-LBRs compared to their female partners (64.4% vs. 58.6%; Paired t-test; p=0.028) and their degree of mis-estimation was also significantly higher (2.3 vs 1.8; Paired t-test; p=0.013). Male and female partners did not differ in level of optimism (Paired t-test; p=0.074) and the correlation between the level optimism and expected IVF-LBR was rather weak (Pearson correlation coefficient in women: 0.428; p=0.000 and in men: 0.254; p=0.038). The correlation between the IVF-prognosis and the level of optimism was also weak (women: Pearson correlation coefficient of 0.022; Men: Pearson correlation coefficient of -0.163).
Limitations, reasons for caution: Recruitment is ongoing to end up with a larger scale prospective cohort study with follow-up until the studied IVF-cycle is completed, by achieving a live birth or a negative pregnancy test after the transfer of the last (fresh and frozen) embryo.
Wider implications of the findings: This study documents (the extend of) the interesting overestimation of IVF-LBRs by couples going through IVF. Whether couple's overestimation leads to distress and ultimately IVF-discontinuation, as suggested by qualitative interviews (Peddie et al., 2005), and is associated with actual LBRs will be followed up.
Trial registration number: not applicable

O-186 Sperm donor conception and disclosure to children aged from <1 to 17 years old: a follow-up study of parents attitudes and children reactions to disclosure.

V. Drouineaud[1], M. Benchaib[2], E. Chapel-Lardic[1], N. Mendes[3], J.P. Wolf[1], C. Chalas[1], L. Ferreux[1], D. Rivet-Danon[1], C. Jean[1], C. Patrat[1], O. Blagosklonov[4], A. Condat[3]

[1]Assistance Publique – Hôpitaux de Paris AP- HP Centre- Université de Paris-, Service de Biologie de la Reproduction - CECOS, Paris, France ;
[2]Hôpital Femme Mère Enfant- Hospices Civils de Lyon-, Service de médecine de la reproduction-, Lyon, France ;
[3]Assistance Publique-Hôpitaux de Paris AP-HP- Groupe Hospitalier de La Pitié-Salpétrière-, Service de Psychiatrie de l'enfant et de l'adolescent-, Paris, France ;
[4]CHU Minjoz, CECOS Franche-Comté - Service de Biologie et Medecine de la Reproduction - Cryobiologie-, Besançon, France

Study question: When do heterosexual parents disclose their use of sperm donation to their child according to the age?
Summary answer: 45.1% of children aged from <1 to 17 years old were informed the day of inclusion. 51.35% were informed between 0-2 and 40.54% between 3-5.
What is known already: In France, gamete donation is free, voluntary and anonymous since 1994. The Bioethics Law does not give any indication concerning the attitude with regard to the disclosure to the child conceived with donor gamete. In the past, couples conceiving through gamete donation were adviced to maintain secrecy. For many years, centers for the study and preservation of eggs and sperm (CECOS) invite couples before ART to disclose to their future child about their origin ton ensure trust. A previous 10-year retrospective follow-up French study reported that 38% of the couples disclosed to the child.
Study design, size, duration: A retrospective follow-up study was performed. Parents with 82 children conceived with donor sperm and aged from 1 to 17 years old were included.
Participants/materials, setting, methods: Parents of 82 children were asked questions of quantitative and qualitative nature about decisions towards their child and other people about their use of donated sperm, how they disclosed and children reactions to disclosure. Five groups were analysed according to the children age: 0-2; 3-5; 6-10; 11-14 and 15-17 years old. The study was set in two centers for the study and preservation of eggs and sperm (CECOS): APHP, Cochin, Paris and Besançon.
Main results and the role of chance: 45.1% of children aged from <1 to 17 years old were informed the day of inclusion: 21.05% of the children aged

0-2 , 45.00% of those aged 3-5, 52.38% of those aged 6-10, 52.94% of those aged 11-14 and 80.00% of those aged 15-17.

51.35% of the children were informed between 0 and 2 years old, 40.54% between 3-5, 8.11% between 6-10, 0% between 11-17. In 54.05% of the cases, both parents disclosed to the child while 2.70% did it separately. Most of the parents who informed their child had informed their family and/or friends about gamete donation before ART (94.59%) and 5.41% after the birth. Although 73.17% couples intended to inform their children about gamete donation before conception, 58.33% disclosed to offspring after birth and 41.67% kept the origin secret. All parents disclosed to their child in a voluntary way. 35.13% used a book and 40.54% told a story and used a book simultaneously. After disclosure, the reactions of the child were different according to the age: indifference (50%) and curiosity (45%) were the most observed in children aged 0-2 while 50% of the kids aged 3-5 showed indifference and/or accepted disclosure. Children aged 6-10 were often curious (66%).

Limitations, reasons for caution: Only parents were interviewed in this study and it should be of great interest to query children conceived through sperm donation. Despite the high response rate, rare parents refused to participate in the study, suggesting that they might not be inclined to disclose the donor origin to their child.

Wider implications of the findings: Attitudes moved towards greater openness about the use of donated gametes, probably due to the evolution of mentalities and awareness of the couples by CECOS centers. Psychological counselling of couples is of particular importance before treatment but also after the child has been born.

Trial registration number: NA

O-187 'Doing' kinship: Experiences of non-genetic parenthood of Dutch heterosexual couples using donor sperm treatment

M. Siermann[1], M. Visser[1], A. Schrijvers[1], M. Mochtar[1], T. Gerrits[2]

[1]Academic Medical Centre Amsterdam, Centre for Reproductive Medicine, Amsterdam, The Netherlands ;
[2]University of Amsterdam, Faculty of Social and Behavioural Sciences, Amsterdam, The Netherlands

Study question: How do Dutch heterosexual couples diagnosed with male infertility who achieved parenthood through donor sperm treatment experience non-genetic parenthood?

Summary answer: For participants genetics were both relevant and irrelevant: genetics were irrelevant for parenthood, but highly relevant for creating similarities between father and child.

What is known already: Male infertility can lead to emotional distress, stigma, and questioning one's sense of masculinity. Non-genetic parenthood can lead to perceived difficulties in parenthood and inequality between parents. Furthermore, donor conception may challenge norms on the role of genetics for kinship. There is limited knowledge on how heterosexual couples experience male infertility, non-genetic parenthood and kinship.

Study design, size, duration: We performed a qualitative in-depth interview study between September 2018 and January 2019 with both partners of thirteen Dutch heterosexual couples who conceived after donor sperm treatment. Participants were recruited through the participant list of a previous mixed-methods study of the University of Amsterdam and the Academic Medical Centre Amsterdam on guidance to donors, parents and children.

Participants/materials, setting, methods: We held semi-structured individual interviews with thirteen genetically related mothers and thirteen non-genetically related fathers (M_{age} fathers = 43.4 years, range 36-64 years; M_{age} mothers = 39.2 years, range 33-46 years). Their 26 children (average: 2 children per couple) were between 1 and 13 years old (M_{age} = 5.4 years). Interview questions were based on literature and clinical experiences of experts in the field of donor conception. Interviews were transcribed and analyzed using thematic analysis.

Main results and the role of chance: All parents in the study were 'doing' kinship: they negotiated the importance of nature versus nurture. Most parents (men and women) saw genetics as irrelevant for experiencing parenthood, for bonding with their children, and for the preferred role of the donor in their future lives. Yet, most of them saw genetics of the donor as relevant for generating similarities between father and child. Having the same donor for all children was seen as essential for nearly all of the parents. Based on the donor's genetic bond with their child, some parents (mainly fathers) had fears around the donor's role in the children's lives and the consequences for the position of the non-genetic father. A few parents (mainly mothers) saw genetics as relevant in terms of possible inherited illnesses from the donor.

Limitations, reasons for caution: There is a possible self-selection bias, with mostly people who are open about donor conception and non-genetic parenthood in their own lives being represented in the study.

Wider implications of the findings: This study gives initial insights into how heterosexual couples in the Netherlands diagnosed with male infertility experience non-genetic parenthood. The findings show the importance of undertaking more qualitative and quantitative studies into experiences of non-genetic parenthood, both to improve guidance for donor conception and to increase knowledge on the topic.

Trial registration number: Not applicable

O-188 Mental health in balance of infertility treatment and employment: Japan-Female Employment and Mental health in Assisted reproductive technology (J-FEMA) Study

Y. Ikemoto[1], K. Keiji[1,2], E. Motoki[3], A. Tanaka[4], S. Rikikazu[2,5], K. Nakagawa[2], Y. Sato[6,7], Y. Kuribayashi[5], K. Tomooka[3], Y. Imai[3], A. Ochiai[1], M. Kitade[1], T. Tanigawa[3], A. Itakura[1], S. Takeda[1]

[1]Juntendo University Faculty of Medicine, Obstetrics and Gynecology, Tokyo, Japan ;
[2]Sugiyama Clinic Shinjuku, Obstetrics and Gynecology, Tokyo, Japan ;
[3]Juntendo University Faculty of Medicine, Public Health, Tokyo, Japan ;
[4]Saint Mother Obstetrics and Gynecology Clinic, Institute for ART, Fukuoka, Japan ;
[5]Sugiyama Clinic Marunouchi, Obstetrics & Gynecology, Tokyo, Japan ;
[6]Tatedebari Sato Hospital, Obstetrics & Gynecology, Gunma, Japan ;
[7]Takasaki Art Clinic, Obstetrics & Gynecology, Gunma, Japan

Study question: The aim of the present study was to identify the risk factors associated with severe psychological stress in women undergoing infertility treatments.

Summary answer: Severe psychological stressors in women undergoing infertility treatments include long infertility duration, no childbirth history, low family income, resignation from job, and infertility-related harassment.

What is known already: Infertility is one of the most stressful experiences for couples who desire to have a child. In Japan, women are increasingly getting married at a later age after their prime reproductive age, thereby leading to declining birthrates and increasing proportions of infertile women. The ratio of the employed women has also risen up to 44.5%, which includes more than 70% of the reproductive-aged women. Therefore, the number of working women undergoing infertility treatments has increased. These women face psychological stress not only during their infertility treatments, but also in their jobs.

Study design, size, duration: This was a cross-sectional multi-center study that was performed from August 2018 to December 2018. We conducted a survey by administering anonymous self-report questionnaires to 1727 subjects in four IVF clinics. This study was approved by the local ethics committee.

Participants/materials, setting, methods: The questionnaire consisted of questions on infertility treatments, work/life conditions, and included the Kessler Six-question Psychological Distress Scale (K6) score for the objective analysis of the psychological distress. Of the 1727 subjects, 1672 subjects were recruited for the analysis; among the excluded 55 subjects, including 36 under treatment for mental disorders and 19 with no data of the K6 scores. We also focused on 1335 subjects who were working when they started the infertility treatments.

Main results and the role of chance: The mean age of the 1672 subjects was 37.6 ± 4.8 years, and the mean K6 score (range: 0–24) was 4.8 ± 4.4; 103 women (6.2%) had K6 scores ≥ 13 (K6 high), and they were diagnosed with severe psychological disorders. Multivariate logistic regression analysis showed that "K6 high" is strongly associated with long infertility duration of 2 years or more (odds ratio [OR] 1.874, 95% confidence interval [CI] 1.080–3.253), no experience of childbirth (OR 2.037, 95% CI 1.046–3.969), and low family income with 6.0 million yen (49,000 euro) or less (OR 1.887, 95% CI 1.041–3.418). Among the 1335 subjects who were working when they started the infertility treatments, 266 women (19.9%) had to resign from their jobs for the

infertility treatment. Multiple regression analysis showed that "high K6" is strongly associated with low family income (OR 2.830, 95% CI 1.517–5.278), the experience of resignation from their jobs (OR 2.081, 95% CI 1.045–4.144), infertility-related harassment at the workplace (OR 2.074, 95% CI 1.080–3.983), and difficulty in working during the infertility treatments (OR 2.938, 95% CI 1.150–7.504).
Limitations, reasons for caution: The pregnancy outcomes and the time-dependent changes in these women could not be analyzed because we had only conducted the survey in the form of anonymous self-report questionnaires in this cross-sectional study.
Wider implications of the findings: Severe psychological stress is associated with increased risks of infertility and pregnancy loss. Meanwhile, a beneficial effect of mental care on pregnancy loss has been reported. Therefore, a compassionate mental care system should be established and women should be supported for balancing their infertility treatment schedules with their jobs.
Trial registration number: I

SELECTED ORAL COMMUNICATIONS

SESSION 48: RELATING THE RELEVANCE OF BIOMARKERS TO INFERTILITY

07 July 2020 Parallel 6 15:15 - 16:30

O-189 Does higher level of serum folate before ovarian stimulation worsen outcomes of assisted reproductive technologies in normogonadotropic women?

M. Polzikov[1], D. Blinov[2], T. Ushakova[1], Z. Barakhoeva[3], M. Ovchinnikova[4], L. Vovk[5], Y. Fetisova[5], O. Sergeyev[6]

[1]IVFarma LLC, n/a, Moscow, Russia C.I.S. ;
[2]Institute for Preventive and Social Medicine, n/a, Moscow, Russia C.I.S. ;
[3]"AltraVita" Human Reproduction Clinic, n/a, Moscow, Russia C.I.S. ;
[4]Group of companies "Mother and Child", Hospital Lapino, Moscow, Russia C.I.S. ;
[5]Group of companies "Mother and Child", Perinatal Medical Centre PMC, Moscow, Russia C.I.S. ;
[6]Belozersky Institute of Physico-Chemical Biology- Lomonosov Moscow State University, Group of Epigenetic Epidemiology, Moscow, Russia C.I.S.

Study question: To investigate the association of serum folate levels before in vitro fertilization with outcomes of ART in a single ovarian stimulation (OS) GnRH-antagonist protocol.
Summary answer: Using multivariable models we suggested that higher serum folate concentrations in normogonadotropic patients was associated with worse early and clinical ART outcomes.
What is known already: Women of reproductive age are recommended to consume about 400 mg folic acid per day from fortified foods, supplements, or both 2–3 month before conception and during pregnancy. Folic acid supplements with different dosage are commonly used by infertile women which can lead to elevated folate concentration. Study by Murto et al. (2014) has shown that women with unexplained infertility had significantly higher median plasma folate concentrations than fertile women. However Gaskins et al. (2015) found that higher serum concentration of folate before ART treatment was associated with higher live birth among subjects of EARTH study using three treatment protocols.
Study design, size, duration: A sub-analysis of data obtained from multicenter, randomized, embryologist-blinded, parallel-group, therapeutic equivalence study of recombinant FSH. 110 women were enrolled using following inclusion criteria: women aged 20-35 years old, tubal and/or male causes of infertility factors, first or second attempt at IVF/ICSI; 18 ≤ BMI ≤ 30 kg/m^2; FSH <10 IU/l, estradiol level <50 pg/ml; AMH≥1.0 ng/ml. No restrictions for folic acid and other food supplements/vitamins were applied before, during and after OS.
Participants/materials, setting, methods: All subjects underwent OS using a GnRH-antagonist protocol and one ART cycle per women. Over the 5-day fixed-dose regimen, the women received 150 IU/day of follitropin alpha, followed by dose adaptation. Baseline serum folate was measured by using a chemiluminescence assay by Architect system (Abbott, USA). Multivariable generalized linear models were used with Poisson distribution and log link function for adjusted oocyte counts, and a binomial distribution and log link function for adjusted clinical outcomes.
Main results and the role of chance: 110 women had a median serum folate concentration of 20.55 ng/ml (interquartile range: 10.8, 32.9 ng/ml). Age, type of follitropin alpha, ovulation trigger, baseline level of estradiol and LH were not associated with both early and clinical ART outcomes in univariate regression models. For each outcome, we fit a full multivariable model including all covariates with univariate p≤0.10 and reduced to a final model retaining covariates with p < 0.05. Women with higher serum folate had significantly lower total oocytes yield (p =0.08), lower number of matured oocytes (p=0.04) and lower fertilized oocytes (p=0.02) after adjustment for BMI, level of AMH, international normalized ratio, duration of stimulation, dose of FSH, ratio serum calcium/magnesium (additionally for male factor in model of fertilized oocytes). Women in highest quartile of serum folate (>=33.0 ng/ml) had 0.23 odds ratio (95% CI: 0.04, 1.34; p=0.046) for biochemical pregnancy, 0.12 odds ratio (95% CI: 0.02, 0.79; p=0.02) for clinical pregnancy, and 0.1 odds ratio (95% CI: 0.01, 0.7; p=0.03) for live birth rate compared with women in the lowest quartile (<10.8 ng/ml) in models adjusted for red blood cells, level of FSH, ratio serum calcium/magnesium (additionally adjusted for level of antithrombin in model of biochemical pregnancy).
Limitations, reasons for caution: Normogonadotropic non-obese study women aged 20-35 years old were unrestricted in use of different supplements, including folic acid and folate. Further pre-conception large-scale studies with known folate status of both parents, including daily doses, serum folate, homocysteine and hormone levels are needed.
Wider implications of the findings: We suggested that baseline higher serum folate concentration was associated with worse ART outcomes. Our findings might be useful for choose of safe dosage of folate supplementation for both fertile women and women undergoing infertility treatment.
Trial registration number: parental study - NCT03088137 (clinicaltrials.gov)

O-190 The mechanism of action of oxytocin receptor antagonists (OTRan) in ART – a study of nolasiban on biomarkers of uterine receptivity in healthy female volunteers

O. Pohl[1], L. Marchand[1], P. Pierzyński[2], C. Blockeel[3], S. Mackens[3], U. Lorch[4], J.P. Gotteland[1]

[1]ObsEva SA, Research and Development, Plan-les-Ouates Geneva, Switzerland ;
[2]Uniwersytecki Szpital Kliniczny, Klinika Rozrodczości i Endokrynologii Ginekologicznej, Białystok, Poland ;
[3]Universitair Ziekenhuis Brussel, Centre for Reproductive Medicine, Brussels, Belgium ;
[4]St George's University of London, Richmond Pharmacology Limited, London, United Kingdom

Study question: How does nolasiban (an OTRan) effect markers of endometrial receptivity such as uterine contractions, and endometrial perfusion and gene expression.
Summary answer: Administration of nolasiban resulted in decreased uterine contractions, increased endometrial blood flow, and modulation of gene expression relevant for endometrial receptivity and implantation.
What is known already: The rate of uterine contractions during embryo transfer (ET) has been shown to be negatively correlated with pregnancy rates, and endometrial blood flow (perfusion) has been shown to be an important predictive parameter for endometrial receptivity. Oxytocin receptors are expressed during human peri-implantation phase in the myometrium, endometrium and uterine blood vessels, and several clinical studies have shown that blocking activation of oxytocin receptors by an OTRan, has the potential to decrease uterine contractions, increase endometrial perfusion and improve pregnancy rates following ET. The highest dose of nolasiban tested in clinical trials is a single administration of 900mg.
Study design, size, duration: A randomized, double-blind, parallel-group study was conducted in 42 healthy, pre-menopausal women aged 18 to 37 years. Following hormonal pre-treatment, on the day corresponding to a Day 5 ET, women received a single oral administration of 900mg or 1800mg nolasiban or matching placebo. Pharmacodynamic assessments up to 24 hours after treatment included ultrasonographic measurements of uterine contraction frequency and endometrial perfusion. Endometrial biopsies were collected at 24 hours.

Participants/materials, setting, methods: Subjects were pre-treated with an identical hormonal preparation as that used for women undergoing frozen-thawed ET (FET). Transvaginal echography at 0, 4, 8 and 24 hours was used to measure uterine contraction frequency, and endometrial perfusion parameters, including vascularity index (VI), flow index (FI) and vascularity flow index (VFI), were measured via 3D power Doppler. Endometrial tissue mRNA expression was analyzed using Next Generation Sequencing with standard analytical methodologies.

Main results and the role of chance: Both 900mg and 1800mg doses of nolasiban had measurable and durable effects on uterine contractions, with a similar decrease in median contraction frequency at 24-hours after treatment. At 1800mg, effects were observed from the 4-hour time point onwards and in post-hoc statistical analyses were at $p<0.10$ at the 4- and 8-hour time points compared to placebo.

Endometrial perfusion parameters showed marked and sustained median increases and were comparable for both doses. The most noticeable increase in VI was between the 8-hour and the 24-hour time points with $p<0.10$ at the 8-hour time point at 1800mg. FI also increased over time and post hoc statistics showed $p<0.10$ at the 24-hour time point for both doses. Similarly, an appreciable increase in endometrial VFI over time was seen at 900mg and 1800mg compared to placebo; $p<0.10$ at the 8-hour time point at 1800mg.

Within 24 hours of 1800mg nolasiban administration, 10 endometrial genes were found to be significantly differentially expressed (adjusted $p<0.05$). Of these, OLFM4, DPP4 and CXCL12 were regulated in the same direction as Window-of-Implantation-associated genes. In addition, three genes (DPP4, CXCL12 and IDO2) have previously been associated with decidualization and endometrial receptivity.

Limitations, reasons for caution: This study was conducted in healthy volunteers without a diagnosis of infertility but pre-treated as for FET. Extrapolation of these results to patients with infertility who are undergoing fresh embryo transfer should be made with caution.

Wider implications of the findings: These data expand the understanding of the mechanism-of-action of OTRan in increasing live birth rates following ET. These results also suggest faster, broader and more potent effects of the nolasiban 1800mg dose compared to the 900mg dose, supporting testing at higher doses and potential alternate regimens in IVF patients.

Trial registration number: 2018-003702-36

O-191 TRIM28 induces follicular arrest and hyperandrogenism

Y. Liu[1], X. Li[1], J. Xu[1], H. Huang[1]

[1]The International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Study question: What is the expression pattern of TRIM28 in granulosa cells(GCs) and the role of that in the mechanism of polycystic ovary syndrome?

Summary answer: The elevated TRIM28 levels reduce ERK phosphorylation by upregulating PDE10A, leading to ovarian steroidogenesis dysfunction in GCs and resulting in follicular arrest and hyperandrogenism.

What is known already: Polycystic ovary syndrome (PCOS) is a common endocrinopathy of unknown etiology that affects women of reproductive age. Previous studies have built a convincing argument that an abnormal AR is associated with PCOS and have documented the unique roles of Androgen receptor(AR) Splicing variants(ASVs)) in GCs in the pathogenesis of PCOS. The AR ASV exhibited a genome recruitment pattern differing from that of wild-type AR and dramatically induced the expression of androgen-related genes. Bioinformatics analysis suggested that this abnormal genomic recruitment pattern of AR ASVs is highly similar to that of multiple transcription factors, of which TRIM28 ranks first.

Study design, size, duration: We compared the gene expression pattern of TRIM28 in granulosa cells(GCs) from 139 PCOS women and 120 women with tubal blockage undergoing IVF-ET between June 2014 and July 2018. We used a human granulosa-like tumor cell line(KGN) to explore the molecular mechanism at the in vitro level. We observed the phenotype of animal modes for 20 weeks.

Participants/materials, setting, methods: 139 PCOS women and 120 women with tubal blockage undergoing IVF-ET in our center were recruited. GCs and follicular fluids were collected during oocyte retrieval. KGN cell line was used for in vitro test. The ovarian specific overexpression of Trim28 transgenicm mice were used as animal models. The phenotypic observation lasted for 20 weeks to clarify the role of Trim28 in the pathophysiological changes of PCOS and explore the molecular mechanism involved.

Main results and the role of chance: We found that the TRIM28 in the granulosa cells (GCs) of women with PCOS were significantly elevated compared with those in the control group ($P<0.05$). We modelled our clinical findings by transgenic mice with ovarian-specific overexpression of Trim28 and followed a PCOS-like phenotype of metabolic abnormalities, including obesity, insulin resistance, hyperandrogenism and follicular dysplasia. Transcriptome analysis of KGN cells revealed that a series of genes were significantly enriched in the ovarian steroidogenesis pathway, and the combination analysis of RNA and ChIP sequences revealed that the mRNA expression of PDE10A was significantly upregulated via TRIM28 in GCs of PCOS women as well as in KGN cells with TRIM28 overexpression, compared with that in the control group ($P<0.05$). Since PDE10A hydrolyzes cyclic adenosine monophosphate (cAMP), the cAMP and PKA activation in TRIM28-overexpressing KGN cells were inhibited, which further led to the inhibition of the MAPK-PKA-ERK signaling pathway and ultimately caused steroidogenesis. Our study thus suggests that elevated TRIM28 levels reduce ERK phosphorylation by upregulating the expression of PDE10A, leading to ovarian steroidogenesis dysfunction in GCs and resulting in follicular arrest and hyperandrogenism, and further confirms the critical role of TRIM28 in the pathophysiological changes of PCOS at the in vivo level.

Limitations, reasons for caution: This study still has certain limitations. We did not explore the reasons for the increased TRIM28 in GCs of PCOS patients, for example, whether it was caused by genetic factors or epigenetic factors.

Wider implications of the findings: Our study first clarified the regulatory mechanism of TRIM28 for PDE10A and their role in the multiple phenotypic interpretations of PCOS and successfully constructed the Trim28-ovarian specific overexpression animal models. The above findings provide new basis and guidance for proposing feasible and effective risk prediction programs and clinical intervention measures.

Trial registration number: 2017YFC1001303

O-192 Androgens increase accumulation of advanced glycation end products (AGEs) in granulosa cells by activating endoplasmic reticulum (ER) stress in polycystic ovary syndrome (PCOS)

M. Harada[1], J. Azhary, MK[1], C. Kunitomi[1], A. Kusamoto[1], N. Takahashi[1], E. Nose[1], N. Oi[1], O. Wada-Hiraike[1], Y. Urata[1], T. Fujii[1], Y. Osuga[1]

[1]Faculty of Medicine- University of Tokyo, Dept. of Obstetrics and Gynecology, Tokyo, Japan

Study question: What is the underlying mechanism regulating AGE – receptor for AGEs (RAGE) system in granulosa cells from PCOS patients?

Summary answer: Targeting AGE-RAGE system, that is upregulated in PCOS ovary and induced by testosterone via activation of ER stress, improves reproductive phenotype of PCOS.

What is known already: PCOS is associated with hyperandrogenism. Previously, we demonstrated that androgen activates ER stress in granulosa cells from PCOS patients and mice, and that this contributes to the pathology of PCOS, including ovarian fibrosis and the growth arrest of antral follicles. In addition, recent studies demonstrated the accumulation of AGEs in granulosa cells from PCOS patients contribute to the pathology by affecting a number of cellular processes, such as steroidogenesis, glucose metabolism, and the production of proinflammatory cytokines.

Study design, size, duration: The expression of RAGE was compared between human granulosa-lutein cells (GLCs) obtained from 11 PCOS patients and ten control patients undergoing oocyte retrieval. Ovaries of three PCOS patients and three control patients, harvested at surgery, were evaluated immunohistochemically for AGEs and RAGE. For *in vitro* experiments, GLCs were obtained from pooled follicular fluids. For *in vivo* experiments, a dehydroepiandrosterone (DHEA)-induced PCOS mouse model was used and forty mice were allocated to four treatment groups.

Participants/materials, setting, methods: Human GLCs were incubated with testosterone and RAGE expression and AGEs accumulation were examined, while GLCs were preincubated with an ER stress inhibitor to examine the intermediary role of ER stress. RAGE expression and AGEs accumulation

in human GLCs and ovaries were compared between control and PCOS patients. PCOS mice were treated with an ER stress or RAGE inhibitor, followed by examination of vaginal smears and quantification of atretic antral follicles.

Main results and the role of chance: Treatment with testosterone increased RAGE mRNA and protein expression in cultured human GLCs. Pre-incubation with a clinically available ER stress inhibitor tauroursodeoxycholic acid (TUDCA) abrogated the stimulatory effect of testosterone on RAGE expression. Treatment with testosterone increased AGEs accumulation in GLCs, and this was reduced by pre-treatment with TUDCA, similar to the pattern for RAGE expression. When we knocked down C/EBP homologous protein (CHOP), a transcription factor activated during ER stress, by RNA interference, CHOP-deficient cells demonstrated significantly lower expression of RAGE expression following testosterone treatment, than control siRNA-treated cells, with a concomitant reduction in AGEs accumulation. GLCs harvested from PCOS patients exhibited higher expression of RAGE mRNA and protein than those from control participants. Immunohistochemical analysis revealed that RAGE expression and AGEs accumulation were increased in granulosa cells of the ovaries from PCOS patients. Increased RAGE expression and AGEs accumulation in granulosa cells of PCOS subjects were further confirmed in PCOS model mice. The administration of a RAGE inhibitor (FPS-ZM1) or TUDCA to PCOS mice reduced the expression of RAGE and the accumulation of AGEs in granulosa cells, and this was accompanied by an improvement in estrous cycling and a reduction in the number of atretic antral follicles.

Limitations, reasons for caution: It is not determined whether the concentrations of testosterone usually utilized in human GLC culture studies are appropriate as a model of *in vivo* hyperandrogenism. Furthermore, the concentrations of testosterone in follicular fluid of antral follicles of PCOS women, not of preovulatory follicles, are still unknown.

Wider implications of the findings: Our findings suggest that the hyperandrogenism of PCOS increases the accumulation of AGEs in the ovary by activating ER stress, and that targeting of the AGE–RAGE system, either by using a RAGE inhibitor or a clinically available ER stress inhibitor, may provide a novel therapeutic approach for PCOS.

Trial registration number: Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (19k09749 , 19k24021., 19k24045, 19h00319 .), a grant from the Japan Agency for Medical Research and Development (18gk0110014h003)

O-193 Polycystic ovary syndrome and leukocyte telomere length: results of cross-sectional and longitudinal analyses in a birth cohort study

P. Pinola[1], J. Pölönen[1], J. Ronkainen[2], A. Blakemore[3], J. Buxton[4], M.R. Järvelin[5], J. Tapanainen[6], S. Franks[7], T. Piltonen[1], S. Sebert[2], L. Morin-Papunen[1]

[1]Oulu University Hospital- University of Oulu, Department of Obstetrics and Gynaecology- Medical Research Center and PEDEGO, Oulu, Finland ;
[2]University of Oulu, Center for Life Course Health Research and Biocenter Oulu, Oulu, Finland ;
[3]College of Health and Life Sciences- Brunel University London and Imperial College London, Department of Life Sciences and Department of Medicine, London, United Kingdom ;
[4]Kingston University London, Department of Biomolecular Sciences- School of Life Sciences- Pharmacy and Chemistry, London, United Kingdom ;
[5]Imperial College London and University of Oulu, MRC-PHE Centre for Environment and Health- Department of Epidemiology and Biostatistics- School of Public Health and Center for Life Course Health Research- Faculty of Medicine, London and Oulu, United Ki ;
[6]University of Helsinki and Helsinki University Hospital, Department of Obstetrics and Gynecology, Helsinki, Finland ;
[7]Imperial College London, Institute of Reproductive and Developmental Biology, London, United Kingdom

Study question: Does mean relative leukocyte telomere length (LTL) or longitudinal change in LTL differ in women with PCOS symptoms or diagnosis in comparison to controls?

Summary answer: In our dataset, women with PCOS do not have shorter LTL, but do show less telomere attrition between ages 31 and 46.

What is known already: Telomeres are DNA-protein complexes that protect chromosome ends from DNA damage and act as a indicator of cellular ageing. In most somatic tissues, a small part of telomeric DNA is lost with every cell division, causing telomere shortening. Current evidence, almost entirely from cross-sectional observations, supports negative associations between LTL and adverse lifestyle and cardio-metabolic risk factors. Women with PCOS are affected by co-morbid conditions that are also associated with shorter LTL, such as obesity and chronic inflammation. To date, few studies have investigated the association between PCOS and LTL and/or LTL shortening with age.

Study design, size, duration: We used a community-based birth cohort (N=5889 women) with clinical follow-up at ages 31 (N=3115) and 46 (N=3280) including measures of LTL at both ages (N=2906 and N=3233). We defined study groups as follows: age 31, both oligo/amenorrhea and hirsutism: "both symptoms" (N=73); age 46, self-reported PCOS diagnosis (DG-PCOS, N=155); self-reported PCOS (both symptoms and/or DG-PCOS: srPCOS, N=207); controls: women without PCOS-related symptoms or diagnosis (age 31: N=1054; age 46: N=1324).

Participants/materials, setting, methods: Mean relative LTL was measured by monochrome multiplex quantitative PCR. The amount of telomeric DNA sequence was quantified relative to a single-copy gene and normalised using a common reference DNA sample. The possible association between LTL and PCOS was analyzed using linear regression models adjusted at both ages for BMI, smoking, alcohol consumption and socioeconomic status. Pearson correlations between LTL and BMI, waist, hip, testosterone and sex hormone-binding globulin were performed at both ages.

Main results and the role of chance: Since LTL was measured using a different reference DNA sample at ages 31 and 46, this was standardized [z score= (LTL–mean (LTL))/SD(LTL)] separately for each measurement.

Unadjusted mean LTL did not differ between women with PCOS symptoms and controls at age 31 (0.01±(SD)1.10 *vs*. 0.07±0.99, P=0.632) or between DG-PCOS women and controls at age 46 (0.03±0.93 *vs*. 0.01±1.02, P=0.860). Similarly, LTL did not differ when comparing srPCOS with controls (at age 31 for srPCOS 0.02±0.99 *vs*. controls 0.07±0.99, P=0.593; at age 46 for srPCOS -0.05±0.95 *vs*. controls 0.01±1.02, P=0.414). After respective adjustments for the aforementioned covariates at age 31 or 46, none of the results changed.

Interestingly, we observed a greater mean difference (MD) of LTL z-scores between ages 46 and 31 in the control group (MD: -0.13±1.10, P<0.001) than in the srPCOS group (MD: -0.01±1.10, P=0.958). The observed difference suggests that the longitudinal change in LTL is markedly less in women with PCOS when compared to controls.

In the srPCOS group there were only weak negative correlations between LTL and BMI (r=-0.17, P=0.019), and LTL and waist circumference (r=-0.19, P=0.009) at age 31 whereas the correlations did not reach significance at age 46.

Limitations, reasons for caution: The symptoms and diagnosis of PCOS were self-reported. The questionnaire at age 46 did not differentiate between women with polycystic ovaries and the syndrome. The study population was relatively young to express significant telomere shortening. The sample size of the PCOS women with telomere measurement data was relatively small.

Wider implications of the findings: We observed less LTL attrition in PCOS women than in controls. Analyses included correction for BMI and lifestyle factors, but before concluding whether there is a causal association between PCOS and LTL, further research is needed to clarify the underlying mechanisms and compare with data from other biomarkers of ageing.

Trial registration number: NA

SELECTED ORAL COMMUNICATIONS
SESSION 49: EMBRYO METABOLISM AND DEVELOPMENT
07 July 2020 Parallel 1 17:00 - 18:00

O-194 Human blastocysts show distinct changes in their metabolism between day 5 and 6

D. Sakkas[1], M. Venturas[2], J.S. Shah[1], T. Sanchez[2], D.J. Needleman[2]

[1]Boston IVF, Research, Waltham- MA, U.S.A. ;
[2]Harvard University, Molecular and Cellular Biology and School of Engineering and Applied Sciences, Cambridge, U.S.A.

Study question: Do day 5 and 6 human blastocysts exhibit metabolic changes that can be detected by non-invasive metabolic imaging?
Summary answer: Imaging of NADH and FAD reveals metabolic differences between day 5 and day 6 discarded human blastocysts.
What is known already: Based on their functional role in cell biology, intracellular NADH and FAD have a diagnostic potential as natural biomarkers for cellular redox reactions and energy metabolism. NADH and FAD are auto-fluorescent, and their concentration and local environment can be measured using Fluorescence Lifetime Imaging Microscopy (FLIM). It has previously been shown that FLIM can quantitatively measure distinct stage differences in the metabolic state of preimplantation mouse embryos over the course of development. Human embryos are known to undergo metabolic changes during the 8-16 cell stage however less is understood of the metabolic idiosyncrasies during the later stages of preimplantation.
Study design, size, duration: We performed a prospective observational study with 88 day 5 and 68 day 6 blastocysts (108 patients, mean age 35.3 years). Blastocysts were discarded and donated for research under an approved institutional review board protocol . All, but 2, embryos had a Gardner score of A and/or B for the inner cell mass and trophectoderm and were either full or expanded blastocysts. The vitrified blastocysts were thawed and cultured for 2 hours prior to imaging.
Participants/materials, setting, methods: A FLIM measurement provides 9 metabolic parameters which can be used to analyze the metabolic signatures of individual embryos. We imaged each blastocyst at three different focal planes, enabling three metabolic measurements per embryo. We then compared those metabolic signatures in relation to the day of vitrification of the embryo (day 5 or 6), using multilevel models.
Main results and the role of chance: FLIM was used to compare the metabolic differences between day 5 and 6 discarded human blastocysts. Of the nine metabolic parameters obtained from each blastocyst the 4 for NADH showed no significant difference (P>0.05) when comparing the Day 5 and 6 blastocysts: NADH short (t1) and long (t2) fluorescence lifetime, fluorescence intensity (I), and fraction of the molecule engaged with enzyme (F). In contrast, all 4 FAD parameters showed highly significant differences (P<0.001) between the Day 5 and 6 blastocysts: FAD-t1; FAD-t2, FAD-I and FAD-F. The redox ratio (Intensity of NADH)/(intensity of FAD), was also compared between Day 5 and 6 blastocysts and showed a substantial significant difference (P<0.001). Subsequent analysis comparing Day 5 and 6 blastocysts with similar expansion rates and similar Gardner grade inner cell mass and/or trophectoderm showed the same pattern.
Limitations, reasons for caution: The study was limited by the type of human blastocysts available as they were discarded and donated for research without any knowledge of their clinical outcome. It would also be expected that the blastocysts would be a mixture of euploid and aneuploid.
Wider implications of the findings: Metabolic imaging can non-invasively detect a significant shift in metabolism between Day 5 and 6 human blastocysts. This analysis indicated that Day 5 and 6 blastocysts may be undergoing intricate changes in respiration as they ready for implantation. Further studies examining metabolic signature over time may provide more information.
Trial registration number: Not applicable

O-195 NMR metabolomics study of follicular fluids reveals specific signature correlated to ART data in endometriosis patients.

K. Pocate[1], P. Santulli[2], F. Kateb[3], M. Bourdon[2], C. Maignien[2], C. Patrat[1], J.P. Wolf[1], G. Bertho[3], C. Chapron[2]

[1]Hopital Cochin, Department of Histology- Embryology- and Biology of Reproduction, Paris, France ;
[2]Hopital Cochin, Gyneacology-Obstetrics and Reproduction clinical unit, Paris, France ;
[3]Paris Descartes University, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques- UMR 8601-CNRS- Université Paris Descartes- ComUE Sorbonne Paris Cité- 45 rue des Saint-Pères- 75006- Paris- France, Paris, France

Study question: To evaluate the relation between metabolic follicular fluid (FF) composition and ART outcomes in terms of embryo quality and live birth occurrence in endometriosis patients.
Summary answer: Embryo quality and live birth occurrence seem to be correlated to a specific metabolic signature in endometriosis patients.
What is known already: Among different mechanisms explaining infertility occurrence in endometriosis, oocyte quality alteration has been raised. This last point is still under debate despite of recent studies confirming the deleterious impact of endometriosis on the oocyte competence. Metabolomics studies are increasingly being used to measure alterations of metabolic pathways associated with complex disorders. Among the various sources of study samples in endometriosis, FF is one of the most interesting to analyze in a context of infertility. Very few studies have focused on the metabolic signature of endometriosis FF and its correlation with ART outcomes.
Study design, size, duration: This is a prospective study started on February 2018 and still recruiting (ClinicalTrials.gov NCT03241329). A total of 9 endometriotic patients were included for this analysis.
Participants/materials, setting, methods: Endometriotic patients enrolled in an ART attempt without any male infertility associated factor. After controlled ovarian stimulation, oocyte retrieval was performed by transvaginal aspiration under ultrasound guidance. Each follicle was individually aspirated and only unique oocyte deriving from the same follicle was retained for the evaluation. FF was then centrifuged and stored at -80°C before 1D-^{1}H NMR-based metabolomics analysis. Data were compared to blastocyst quality assessment and to clinical outcome after transfer.
Main results and the role of chance: Analysis was carried out on a total of 26 FF samples for which embryo quality was assessed and on 8 FF samples for which clinical outcome information was available. Univariate analysis revealed that the concentration of various statistically relevant metabolites could be associated with embryo quality. In particular, the metabolic profile of FF resulting in better embryo quality was characterized by a decrease in glucose level associated to an increase in lactate level. Among other discriminant metabolites, citrate, acetate and amino acids, such as tyrosine also appear as being particularly relevant with a lower concentration level observed for good embryos. Finally, live birth occurrence appears to be correlated to the same trend of variation in glucose and lactate levels, but also to an increase in lipids levels such as HDL cholesterol, 2-octenoate and alkane.
Limitations, reasons for caution: Even if the methodology of our study was robust, the analysis was realized using a small number of samples. These preliminary results need to be confirmed on larger series.
Wider implications of the findings: Embryo quality and live birth occurrence seem to be correlated to a specific metabolic signature in endometriosis patients. Our results could be linked to the oocyte quality and could then represent a new and non-invasive tool for the prediction of succeeding chance in pregnancy favorable outcomes.
Trial registration number: not applicable

O-196 Blastocyst quality modulates the generation of an inflammatory microenvironment by decidualized cells

L. Fernandez[1], E. Grasso[1], E. Soczewski[1], M.S. Gori[1], G. Calo[1], V. Hauk[1], G. Martinez[2], M. Irigoyen[2], C. Perez Leiros[1], R. Ramhorst[1]

[1]CONICET. Universidad de Buenos Aires., Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales IQUIBICEN. Laboratorio de Inmunofarmacología y Laboratorio de Inmunología Reproductiva de la Fertilidad., Buenos Aires, Argentina ;
[2]Fertilis Medicina Reproductiva, Laboratorio de embriologia, San Isidro, Argentina

Study question: Could soluble factors secreted by blastocyst (blastocyst conditioned media or BCM) control the inflammatory response during the peri-implantation period according to blastocyst quality?
Summary answer: Decidualized cells increased the expression/production of inflammatory cytokines as well as neutrophils migration and activation in response to BCM from impaired development (ID) blastocyst.

What is known already: The decidualization program in humans starts on each menstrual cycle and implies not only phenotypical changes on the endometrial stromal cells, but also in their secretory profile. This secretome includes pro-implantatory factors as well as pro and anti-inflammatory cytokines. Despite blastocyst implantation needs a sterile inflammatory response, it should be later controlled in favour of a tolerogenic microenvironment. In this sense, decidualized cells display the ability to change their secretome according to the quality of the blastocyst.
Study design, size, duration: Human endometrial stromal cell line (HESC) was decidualized with medroxiprogesterona and dbcAMP during 8 days. Then, decidualized HESC cells were stimulated with human blastocyst conditioned media (BCM) obtained from developing blastocysts (normal development or ND) or arrested ones (impaired development or ID). Non decidualized cells were used as control.
Participants/materials, setting, methods: BCM were recovered from 5 days individually cultured blastocyst obtained from IVF/ICSI and classified as normal or impaired development (ND/ID) according to Istanbul consensus. Neutrophils were obtained from peripheral blood from healthy donors. Cytokine expression/production was evaluated by RT-qPCR/flow cytometry/ELISA. Caspase-1 activity was measured using Flica probe. Neutrophils migration towards HESC supernatants was evaluated using a transwell system. ROS production was assessed by CDFH-DA probe. MMPs activity was evaluated by gelatin zymography.
Main results and the role of chance: We observed that ID-BCM stimulation increased caspase-1 activation and IL-1β production by decidualized cells, while ND-BCM reduced IL-1β production ($p<0.05$, ANOVA). When a cytokine profile was evaluated, we observed that ID-BCM increased CCL2 and CXCL12 expression as well as IL-8 secretion, while the production of the anti inflammatory cytokine IL-10 was reduced ($p<0.05$, ANOVA). In contrast, ND-BCM stimulation did not modulate these cytokines. Since IL-8 and CXCL12 are associated to neutrophils recruitment towards the inflammatory sites, we evaluated whether the HESC supernatants were able to modulate neutrophils migration using a transwells system. In this sense, ID-BCM increased neutrophils recruitment. Additionally, when neutrophils were stimulated with HESC supernatants, ID-BCM treatment leaded to higher ROS production ($p<0.05$, Friedman test), suggesting an increased activation of these immune cells. Since menstruation is accompanied by a neutrophils influx, we hypothesize that ID-BCM stimulation induces a "menstruation like" pro-inflammatory microenvironment. Due to the fact that MMPs are the final effectors of menstruation, we tested MMPs activity on HESC conditioned media. In this sense, ID-BCM stimulation increased geletinolitic activity of MMP9 ($p<0.05$, ANOVA).
Limitations, reasons for caution: The present results were obtained using immortalized cell lines and *in vitro* models. Further studies are necessary to elucidate whether the mechanisms operate similarly *in vivo* and rule out any factor not contemplated *in vitro*.
Wider implications of the findings: These results provide new clues about the embryo maternal crosstalk and the natural embryo selection. Decidualized cells respond to blastocyst derived factors according to their quality either controlling or inducing a pro-inflamatory microenvironment. These findings might contribute to a better understanding of reproductive disorders such as *in vitro* implantation failures.
Trial registration number: not applicable

O-197 What can we learn from the first 24 hours of embryo development? A fully automated AI-based algorithm for identifying high-quality blastocysts.

D. Gilboa[1], M. Meseguer[2], R. Maor[1], R. Weiner[1], L. Alon[1], L. Bori[2], L. Alegre[2], D.S. Seidman[3]

[1]AiVF, IVF Research and Development, Tel Aviv, Israel ;
[2]IVIRMA Global, IVF Unit, Valencia, Spain ;
[3]Sheba Medical Center, IVF Unit, Ramat Gan, Israel

Study question: Can an AI-based system that analyses the first 24 hours of embryonic development open, for the first time, a window to the development of the embryo?
Summary answer: Using AI algorithms we have shown that a combination of previously unrecognized features within 24 hours after fertilization can help identify high-quality blastocyst
What is known already: Embryos examined on the first day after retrieval are usually classified as viable or atretic. However, little more can be established regarding their future development, based on routine morphological studies. All AI algorithms developed so far for embryo selection are based on time-lapse video analysis of cleavage stage embryos. Oocyte and zygote stage visual assessment was not used so far for the prediction of embryo quality.
Study design, size, duration: A retrospective analysis of the first 24 hours of embryo development based on 1560 consecutive time-lapse videos from a single IVF center.
Participants/materials, setting, methods: Using computer vision algorithms, we identified unique features, some of which were never before recognized, during the first 24 hours of embryonic development. We then developed an automated AI algorithm to extract and measure these features from time-lapse videos. A deep neural network was developed based on the parameters recognized by the computer vision module for each image. A classifier was used to match these parameters to the endpoint, high-quality blastocysts.
Main results and the role of chance: Our model was able to predict high-grade blastocysts on day 5 using only day-1 time-lapse data with an AUC of 0.665 [95% CI 0.650-0.681] and 10-fold stratified cross-validation of the training set. Our test results showed that the AUC was reproducible. These results are comparable to studies attempting to make similar predictions based on a much longer observation period of cleavage stage embryos.
Limitations, reasons for caution: Future prospective validation of our AI algorithm is required using different patient populations, although the number of embryos analyzed is outstanding.
Wider implications of the findings: Our results broaden the understanding of the potential capabilities for IVF of newly introduced AI systems. We showed that a deep neural net can solve one of the most challenging tasks, distinguishing between top-grade embryos, solely based on unique visual features identified in the first 24 hours after fertilization.
Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 50: ANDROGEN TREATMENT IN FERTILITY MANAGEMENT

07 July 2020 Parallel 2 17:00 - 18:00

O-198 A prospective study of testosterone gel treatment in poor ovarian reserve in IVF-ICSI cycles

R. Singh[1], M. Singh[2]

[1]BHOPAL TEST TUBE BABY CENTRE, INFERTILITY, BHOPAL, India ;
[2]BTTB Centre, Infertility, Bhopal, India

Study question: Does transdermal testosterone gel pretreatment improve the outcome in women with poor ovarian reserve undergoing IVF-ICSI Cycles ?
Summary answer: The testosterone gel has a significant impact on the fertility rate in women with a poor response in the IVF cycles.
What is known already: Poor ovarian reserve to external gonadotropin drugs is one of the problems with IVF-ICSI cycles which can lead to cycle stop, access to fewer oocytes and embryos, and finally reduced pregnancy rates.
No effective approach has been found yet to treat poor response to ovarian stimulation.
However, there are possible methods affecting the performance of gonadotropins on the ovaries such as high-dose gonadotropins, growth hormone, glucocorticoids, and low-dose aspirin. Another treatment is the use of low-dose androgens to improve ovarian response to gonadotropins which acts by increasing the intrafollicular androgen and the number of follicle-stimulating hormone (FSH) receptors on granulosa cells.
Study design, size, duration: 52 patients from July 2017 to July 2019, were randomly divided into two groups , 26 patients treated with a

placebo (lubricant gel, control group) and 26 patients treated with testosterone gel (Study group).
Inclusion criteria were : patients for IVF cycles, patients older than 40 years, a cycle with previous poor response, i.e., to obtain 3 or <3 oocytes of the cycles by normal stimulation, AFC <5–7, AMH <0.5–1.1 ng/ml, Fertility outcomes were compared.
Participants/materials, setting, methods: 52 patients were randomly divided into two groups. 26 patients treated with a placebo gel and 26 patients treated with testosterone gel .

Patients who met inclusion (Bologna) criteria were placed in the antagonist cycle group. The patients were randomly divided into two groups each included 26 participants treated with a placebo and testosterone gel . Fertility outcomes were compared between two groups.

The two groups were not statistically different in terms of FSH , AFC, AMH,
Main results and the role of chance: The number of oocytes and embryos in the study (testosterone gel) group were significantly higher than in the control group.

The mean number of oocytes obtained was 3.12 ± 1.14 versus 1.27 ± 1.03 and embryos was 2.10 ± 1.08 versus 0.39 ± 0.48.

The clinical pregnancy rate was 15% (4/26) in the study (testosterone gel) group ,were significantly higher versus than in the control group 04 % (1/26).

In conclusion, there is evidence from this study that the use of transdermal testosterone prior to ovarian stimulation in women who are considered poor responders, and this treatment has shown to significantly improve live birth rates and reduce the doses of FSH required for ovarian stimulation.

Androgen receptors are expressed in granulosa cells at early stages of follicle maturation, it is surprising that such a short treatment up to 20 days of testosterone supplementation could achieve significantly higher live birth rates. Hence, extending testosterone supplementation for a longer period could enhance the pool of follicles sensitive to gonadotrophins and therefore increase the number of oocytes available for retrieval.
Limitations, reasons for caution: Transdermal-testosterone may improve the clinical outcomes for poor-ovarian-reserve.

One limitation is the low number of participants and exact subgroup of poor-ovarian-reserve who would benefit from this treatment still needs to be identified.

Although trends in all parameters appear to favour testosterone supplementation, further investigations are needed to confirm these findings.

Wider implications of the findings: According to the results of our study, the testosterone gel has a positive impact on fertility rate in patients with poor-ovarian-reserve.

The identification of poor responders that could especially benefit from testosterone treatment should be addressed in further studies.

Large studies on larger populations are recommended to be conducted.
Trial registration number: not applicable

O-199 The efficacy and molecular mechanism of dehydroepiandrosterone in diminished ovarian reserve

C. Çakır[1], G. Kuspınar[1], S. Işıklar[1], K. Aslan[2], I. Kasapoglu[2], G. Uncu[2], B. Avcı[1]

[1]Uludag University School of Medicine, Histology and Embryology & Gynecology and Obstetric ART Center, Bursa, Turkey ;
[2]Uludag University School of Medicine, Gynecology and Obstetric ART Center, Bursa, Turkey

Study question: What is the effectiveness and molecular mechanism of dehydroepiandrosterone(DHEA) added to the controlled ovarian hyperstimulation(COH) protocol in diminished ovarian reserve (DOR) in vivo rat model?
Summary answer: DHEA improves ovarian respons by increasing folliculogenesis and suppresses apoptotic process of follicles with modulation of the systemic inflammatory response by reducing COX-2 gene expression.
What is known already: Despite the controversy about its effect and uncertainty about the exact mechanisms, DHEA is added as an adjuvant in over a quarter of IVF treatment protocols in patients with poor ovarian response. DHEA stimulates primordial follicles initiation and preantral follicular development in the gonadotrophin responsive stages. It is believed that DHEA increase ovarian susceptibility to FSH stimulation at the later stage but there has not been an established evidence to support at the follicular/molecular level.
Study design, size, duration: In order to determine the efficacy of DHEA both alone and combination with the COH protocol in the low and normalovarian reserve experimental groups, 75 Spraque Dawley female rats were divided into 15 groups. (Control group, VCD group, VCD+DHEA group, VCD+DHEA+KOH group, VCD+DHEA+SF group, VCD+DMSO group, VCD+DMSO+KOH group, VCD+DMSO+SF group, DMSO group, DMSO+DHEA group, DMSO+DHEA+KOH group, DMSO+DHEA+SF group, DMSO+DMSO group, DMSO+DMSO+KOH group and DMSO+DMSO+SF group).
Participants/materials, setting, methods: The experimental animal model of DOR was composed by 4-Vinylcyclohexene diepoxide (VCD) injections (160mg/kg/day for 15 days). After VCD or DMSO injections, DHEA injections were administered (60mg/kg/day for 45 days). COH protocol was then applied to the subjects. In ovarian tissues obtained from sacrificed subjects, COX-2 gene expression levels were analyzed by RT-qPCR, morphological evaluation and ovarian follicle count were performed by hematoxylin-eosin staining.
Main results and the role of chance: It was determined that VCD injections caused a decrease in the number of follicles at different stages of development ($p<0.05$) and an increase in the number of atretic follicles ($p<0.05$) and COX-2 gene expression levels ($p<0.05$) in the ovarian tissues. While DHEA injections did not affect folliculogenesis and COX-2 gene expression levels in subjects with normal ovarian reserve, it was determined that DHEA injections caused a decrease in atretic follicle counts ($p<0.02$) and an increase in COX-2 gene expression levels in subjects with low ovarian reserve ($p<0.01$). DHEA injections before the COH protocol affected folliculogenesis positively in both low and normal ovarian reserve experimental groups, and thus enhanced the efficiency of the COH protocol. Also, DHEA decreased COX-2 gene expression levels in all subjects($p<0.001$).
Limitations, reasons for caution: The limitations of the study is that clinical outcomes have not been evaluated.
Wider implications of the findings: While evidence of using DHEA in clinical setting is currently uncertain, this study shows that DHEA can modify the ovarian microenvironment and modulate the systemic inflammatory response by reducing COX-2 expression.DHEA treatment potentially may be useful clinically as a means to increase the number of gonadotropin-responsive follicles for ovarian stimulation.
Trial registration number: Not applicable

O-200 Endocrine and histological effects of androgen treatment in female-to-male transsexual patients: a study model of long-term high-dose ovarian androgenization

G. Casals Soler[1], A. Borrás Capó[1], A. Goday Cibeira[1], M. Mora Porta[2], S. Peralta Flores[1], F. Fàbregues Gasol[1], J.M. Calafell Pozo[1], D. Manau Trullàs[1], F. Carmona Herrero[3]

[1]Hospital Clinic de Barcelona, Assisted reproduction unit, Barcelona, Spain ;
[2]Hospital Clinic de Barcelona, Endocrine department, Barcelona, Spain ;
[3]Hospital Clinic de Barcelona, Gynecology department, Barcelona, Spain

Study question: Which are the hormonal and ovarian histological effects of a long-term high-dose androgen treatment using female-to-male transsexual patients (FTMTP) as a study model?
Summary answer: According to this study model, long-term high-dose androgen treatment is associated with sequential preoperative hormonal changes, heterogeneous postoperative endocrine parameters and characteristic ovarian histological features.
What is known already: FTMTP treated with high-doses of androgens for a long period of time constitutes an excellent endocrinological, metabolic and histological study model of ovarian androgenization. The aim of the present investigation is: a) to identify the sequence of endocrinological changes in these patients, from the baseline period prior to the start of androgenization to the postoperative

stage and beyond, with controls up to 6 months after surgery; b) histological ovarian assessment after a long period of treatment with androgens at high doses.
Study design, size, duration: Longitudinal observational prospective study including 60 FTMTP patients treated with long-term high-dose of systemic androgens (testosterone undecanoate, Reandron® 1g every 3 months by intramuscular injection). The study points were three: before starting androgen treatment (point 0); after two years of androgen treatment, performed the day before surgery, which consisted in a laparoscopic hysterectomy and bilateral adnexectomy (point 1); and 6 months after surgery (point 2).
Participants/materials, setting, methods: We performed serum hormonal tests including AMH, FSH, LH, estradiol, androgenic profile (androstenedione, testosterone, SHBG) and fasting insulin in each point of the study. Ultrasonographic scans were performed in 32 patients at point 0 and 1 of study. The histological examination was performed in histological sections stained with hematoxylin and eosin. The stromal structure, the thickness of the *tunica albuginea* and the developmental stages of the follicles were evaluated, counting follicles of each category separately.
Main results and the role of chance: The mean age (±SD) was 27.81±5.81 years old (range 20-34). Five patients presented previous polycystic ovary syndrome (PCOS) criteria, with a transvaginal ultrasonography with ≥20 follicles <10mm, ovarian volume ≥10 mm^3 and oligomenorrhea. All patients became amenorrheic after starting the hormonal treatment. At point 1 of the study, all the patients showed hirsutism, no follicular activity by ultrasonography, high testosterone serum levels (781,5 ng/ml ± 325,9) and other serum hormonal parameters in the reference ranges: AMH (3,23±1,4 ng/ml), FSH (5,95±1,97U/L), LH (4,7±3,45 U/L) and estradiol (46,8±24,9 pg/ml). The histological follicular population study demonstrated 88% of primordial follicles, 3.1% of primary follicles, 0.7% of secondary follicles, 4.9% of antral follicles and 3.4% of atretic follicles. We must highlight a high presence of luteinization of stromal cells (68.5% of cases) and only two cases with stromal hyperplasia (1.8%). The thickness of the tunica albuginea was heterogeneous (mean=0.55±0.22 mm; range 0.15-1.45). These ovarian samples share some characteristics with PCOS ovaries although they do not reproduce their complete histological criteria (Hughesdon, 1982). Hormonal assessment showed high heterogeneity at point 3 of study (mean ± SD; range): FSH 52.0±36.0 U/L (0.4-102.4), LH 26.5±21.59 U/L (0.1-68.3) and estradiol 36.2±7.9 pg/ml (27-45).
Limitations, reasons for caution: The sample size is limited. We could not include a control group of non-androgenized patients due to ethical reasons. Ovarian samples from surgery for other indications did not present adequate characteristics to be considered as controls due to histological changes secondary to different associated pathologies.
Wider implications of the findings: We demonstrated that long-term high-dose systemic treatment with androgens is associated with: a) sequential hormonal changes during the preoperative period and heterogeneous postoperative data, possibly due to a hypothalamus and/or pituitary gland inhibition in a selected group of patients; b) characteristic follicular and stromal changes in the ovarian histological study.
Trial registration number: Not necessary

O-201 Association between levels of testosterone /DHEAS at initiation of ovarian stimulation for in vitro-fertilization and the probability of pregnancy: a systematic review and meta-analysis

V. Christoforaki[1], J. Bosdou[1], G. Lainas[1], T. Lainas[2], A. Kalpatsanidis[1], A. Mouza[1], G. Grimbizis[1], E. Kolibianakis[1]

[1]Aristotle University of Thessaloniki- Medical School, Unit for Human Reproduction- 1st Department of Ob/Gyn, Thessaloniki, Greece ; [2]EUGONIA, Assisted Reproduction Unit, Athens, Greece

Study question: Is the probability of pregnancy associated with levels of testosterone/ dehydroepiandrosterone-sulphate (DHEAS) at initiation of ovarian stimulation for in-vitro fertilization (IVF)?
Summary answer: At initiation of ovarian stimulation, a significant negative association exists between testosterone and live birth, whereas a non-significant association exists between DHEAS and clinical/biochemical pregnancy.
What is known already: It has been shown that androgen action is required for normal follicular growth and progression beyond pre-antral stage. Androgens exert direct autocrine and/or paracrine effects that regulate follicular development, by augmenting follicle stimulating hormone (FSH) receptor expression in granulosa cells. Until today, several studies have examined the association between endogenous androgen and achievement of pregnancy, with conflicting, however, results, that have not been yet systematically reviewed.
Study design, size, duration: A systematic review and meta-analysis was conducted after performing a literature search in MEDLINE, CENTRAL until October 2019. The main outcome was the association between levels of testosterone / DHEAS and the probability of pregnancy. Meta-analysis was conducted by pooling data to calculate standardized mean differences (SMD) with 95% confidence intervals (CI), using random effects model.
Participants/materials, setting, methods: Thirteen eligible studies, including 3977 patients, published between 1992 and 2017 were identified. In these studies, testosterone and DHEAS levels were assessed prior to initiation of stimulation among women who after embryo transfer achieved or not pregnancy. Three out of the thirteen studies were prospective, eight were retrospective and in two studies the design was unclear.
Main results and the role of chance: Inhibition of premature luteinizing hormone (LH) surge was performed using gonadotrophin-releasing-hormone (GnRH) agonists (n=9 studies), GnRH-antagonists (n=2 studies), either GnRH-agonists or antagonists (n=1 study), while this information was not reported in one study. Ovarian stimulation was performed with recombinant-FSH (n=6 studies), urinary FSH (n=4 studies), both recombinant and urinary FSH (n=2 studies), while this information was not reported in one study.

Significantly lower testosterone levels were present in patients who achieved live birth compared to those who did not (SMD: -0.21, 95%CI: -0.38 to -0.04, p=0.02, one study, 731 patients).

Testosterone levels were not significantly different between patients who achieved clinical pregnancy and those who did not (SMD: -0.17, 95%CI: -1.27 to +0.93, p=0.76, eight studies, 2016 patients) as well as between those who had positive pregnancy test compared to those who did not (SMD: -0.16, 95%CI: -0.43 to +0.11, p=0.24, four studies, 1230 patients).

DHEAS levels were not significantly different between patients who achieved clinical pregnancy and those who did not (SMD: +0.05, 95%CI: -0.09 to +0.18, p=0.53, six studies, 942 patients) as well as between those who had positive pregnancy test compared to those who did not (SMD: -0.01, 95%CI: -0.73 to +0.71, p=0.98, one study, 120 patients).
Limitations, reasons for caution: Heterogeneity was present regarding patient population, as well as the type of ovarian stimulation protocol used, which could not be meaningfully explored by subgroup analysis, due to the limited number of eligible studies.
Wider implications of the findings: The current study does not suggest that there is a positive association between testosterone / DHEAS levels at initiation of stimulation and pregnancy achievement, questioning the rationale behind testosterone and DHEAS pre-treatment in patients undergoing ovarian stimulation for IVF.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 51: RM: NEW DIAGNOSTIC AND THERAPEUTIC ASPECTS

07 July 2020 Parallel 3 17:00 - 18:10

O-202 The transcriptomic profile of endometrial receptivity in recurrent miscarriage

L. Craciunas[1], O. Pickering[1], J. Chu[1], M. Choudhary[2], J. Zurauskiene[3], A. Coomarasamy[1]

[1]University of Birmingham, Tommy's National Centre for Miscarriage Research, Birmingham, United Kingdom ; [2]Royal Victoria Infirmary, Assisted Conception Unit, Newcastle, United Kingdom ; [3]University of Birmingham, Centre for Computational Biology, Birmingham, United Kingdom

Study question: What is the transcriptomic profile of the endometrium in women who suffered unexplained recurrent miscarriage and can it predict the fate of the subsequent pregnancy?

Summary answer: Endometrium biopsied from women who suffered high order recurrent miscarriage has a distinct transcriptomic profile and the differences may predict the fate of subsequent pregnancies.

What is known already: Embryo chromosomal abnormalities represent the most common cause of first trimester miscarriage in addition to a broad range of maternal health problems. Genomic studies based on the products of conception reported decreasing rates of chromosome abnormalities as the order of miscarriage increased, with a significant turning point after four or five miscarriages, suggesting the cause of miscarriage more likely to be non-chromosomal in higher order miscarriages. Endometrial transcriptomics aim to describe the full range of RNA transcripts that are produced at the level of the endometrium with an aim to develop a new test of endometrial receptivity.

Study design, size, duration: This was a multicentre cohort study performed in the Tommy's National Centre for Miscarriage Research in Birmingham, Saint Mary's Hospital in Manchester and Royal Devon & Exeter Hospital, United Kingdom. The study was conducted between December 2017 and December 2019. It included 24 women diagnosed with unexplained recurrent miscarriage based on strict inclusion criteria to rule out known causes and risk factors for miscarriage, while increasing the risk of an unidentified endometrial receptivity problem.

Participants/materials, setting, methods: Endometrial biopsies were obtained during implantation window from 18-35 years old women, not pregnant and regularly menstruating, diagnosed with unexplained recurrent miscarriage by undertaking standard investigations according to the ESHRE guidelines. They were excluded for miscarriage risk factors such as smoking, obesity or hyperprolactinemia. The RNA transcripts abundances were quantified using Kallisto. R packages tximport and DESeq2 were used to summarize count estimates at the gene level and to analyse the differential gene expression.

Main results and the role of chance: When compared to controls (two or three miscarriages), women who suffered four or more miscarriages had 19 differently expressed genes after adjustment for multiple comparisons. They were related to biological processes such as immunity (HLA-DMA, CCR8, ALOX5), energy production (ATP12A), hormone secretion (CGA), adhesion (CHAD, ADGRF2, AQP5, TBCD, CTNND1, NKD2) and cell proliferation (NCCRP1).

Important differences in the transcriptomic profiles were identified when women who suffered a subsequent miscarriage were compared to women who had a live birth following the endometrial biopsy. The number of significantly differently expressed genes was 421. Their gene ontology mapped to processes relevant to embryo implantation and early stages of pregnancy such as biosynthetic process (129 genes), signal transduction (124 genes), response to stress (113 genes), immune system process (84 genes), cell differentiation (75 genes), catabolic process (67 genes), cell death (66 genes), cell proliferation (59 genes), homeostatic process (41 genes), cytoskeleton organization (40 genes), cell adhesion (38 genes) or reproduction (26 genes).

Limitations, reasons for caution: This was a first discovery study to evaluate endometrial receptivity using transcriptomics in a relatively small selected population of women who suffered unexplained recurrent miscarriage. The findings reported here have not been validated in an independent sample set yet.

Wider implications of the findings: Abnormal transcriptomic profiles may explain variations in endometrial receptivity and build up on the evidence to support non-chromosomal causes for higher order miscarriages. Validation of the biological changes identified prior to a subsequent miscarriage or live birth may aid in developing a prediction test for use in clinical practice.

Trial registration number: NCT03442335

O-203 Systematic review and meta-analysis of prevalence of microbiota in ART cycles and recurrent pregnancy loss

S. Robati[1], E. Chronopoulou[1], E. Theodorou[1], P. Serhal[2], W. Saab[2], V. Seshadri[2]

[1]Institute for Women's Health- University College London, Faculty of Population Health Sciences, L0ndon, United Kingdom ;
[2]The Centre for Reproductive & Genetic Health CRGH, Population Science and Women's Health, London, United Kingdom

Study question: Does microbiota influence miscarriage rates in ART cycles and contribute to recurrent miscarriage?

Summary answer: This meta-analysis has demonstrated the importance of screening for microbiota, including Bacterial Vaginosis (BV) and Chlamydia Trachomatis (CT).

What is known already: Approximately 15% of pregnancies end up in miscarriage, contributing to one of the most common adverse pregnancy outcomes. Most causes remain unknown, although a complex interplay of different factors are thought to be involved and the presence of infection plays a pivotal role. Microbiota affects all facets of reproduction including pregnancy loss; however, few studies have been conducted to quantify the prevalence of each microbiome following ART (assisted reproductive technology) and its role with recurrent miscarriage.

Study design, size, duration: Systematic review and meta-analysis of published controlled studies. Searches were conducted from January 1999 to June 2019 on MEDLINE and EMBASE using the following search terms: miscarriage OR recurrent miscarriage OR microbiome (bacterial vaginosis (BV), chlamydia, herpes simplex virus (HSV), human papillomavirus (HPV), cytomegalovirus (CMV) and toxoplasma or toxoplasmosis) AND IVF. Studies were limited to those published in English.

Participants/materials, setting, methods: A total of 225 full-text articles were assessed for eligibility from 310 records identified through database searching. 30 studies were included in the quantitative synthesis (meta-analysis). References were based on title and abstract and assessed utilising the Newcastle–Ottowa Quality Assessment Scales.

Main results and the role of chance: For patients undergoing IVF treatment, a significant association was observed with early spontaneous miscarriage for Bacterial Vaginosis (BV) (RR 1.56, 95% CI 1.14–2.21), which supports existing literature. Chlamydia Trachomatis (CT) had two studies which limited clinical significance and Herpes Simplex Virus (HSV) did not show any statistical significance for miscarriage following IVF (RR 1.28, 95% CI 0.85-1.92). There were no studies for the other microbiota. There was no significant relationship between microbiota infection and recurrent miscarriage; however, meta-analysis was carried out for the association between CT and Cytomegalovirus (CMV) with recurrent miscarriage (OR 1.30, 95% CI 0.61-2.78 and OR 2.58, 95% CI 0.84-7.90 respectfully). There were insufficient studies for the remaining microbiota to perform a meta-analysis.

Limitations, reasons for caution: There is a paucity of data for specific micriobiota. Some sample sizes were too small to find any significant association with miscarriage and further study is necessary.

Wider implications of the findings: This study has shown the importance of screening before ART treatment and for women with recurrent miscarriages. There may be a role for specific commensals including Lactobacillus that may improve the intrauterine environment to aid with implantation. Being the first meta-analysis within this field is valuable for patient counselling.

Trial registration number: not applicable

O-204 The metabolomic profile of endometrial receptivity in recurrent miscarriage

L. Craciunas[1], J. Chu[1], O. Pickering[1], L. Mohiyiddeen[2], W. Dunn[3], A. Coomarasamy[1]

[1]University of Birmingham, Tommy's National Centre for Miscarriage Research, Birmingham, United Kingdom ;
[2]Central Manchester University Hospitals, Saint Mary's Hospital, Manchester, United Kingdom ;
[3]University of Birmingham, School of Biosciences, Birmingham, United Kingdom

Study question: What is the metabolomic profile of the endometrium in women who suffered unexplained recurrent miscarriage and can it predict the fate of the subsequent pregnancy?

Summary answer: Endometrium biopsied from women who suffered high order recurrent miscarriage has a distinct metabolomic profile and the differences may predict the fate of subsequent pregnancies.

What is known already: Embryo chromosomal abnormalities represent the most common cause of first trimester miscarriage in addition to a broad range of maternal health problems. Genomic studies based on the products of conception reported decreasing rates of chromosome abnormalities as the order of miscarriage increased, with a significant turning point after four or five miscarriages, suggesting the cause of miscarriage more likely to be non-chromosomal in higher order miscarriages. Endometrial metabolomics aim to describe

the full range of metabolites identified in a tissue sample obtained from the endometrium with an aim to develop a new test of endometrial receptivity.
Study design, size, duration: This was a multicentre cohort study performed in the Tommy's National Centre for Miscarriage Research in Birmingham, Saint Mary's Hospital in Manchester and Royal Devon & Exeter Hospital, United Kingdom. The study was conducted between December 2017 and December 2019. It included 24 women diagnosed with unexplained recurrent miscarriage based on strict inclusion criteria to rule out known causes and risk factors for miscarriage increasing the risk of an unidentified endometrial receptivity problem.
Participants/materials, setting, methods: Endometrial biopsies were obtained during implantation window from 18-35 years old women, not pregnant and regularly menstruating, diagnosed with unexplained recurrent miscarriage by undertaking standard investigations according to the ESHRE guidelines. They were excluded for miscarriage risk factors such as smoking, obesity or hyperprolactinemia. The metabolite composition and relative concentrations of samples were analysed applying ultra-high performance liquid chromatography-mass spectrometry. Raw data were processed applying XCMS and statistical analysis was applied using the software MetaboAnalyst.
Main results and the role of chance: There were distinct metabolomic profiles between the endometrial samples obtained from women with higher order miscarriage defined as more than four recurrent miscarriages and more than five recurrent miscarriages, respectively. More biological differences were identified when the cut-off for the extreme group was set at five miscarriages and the analysis showed groups of metabolites from the same metabolite class, supporting the class-specific changes with more confidence. These groups of metabolites related to cardiolipins, ceramides and glycerophospholipids. Cardiolipins are mitochondrial cell membrane lipids and indicate mitochondrial stress and morphological changes. Ceramides are generally believed to be lipotoxic, while glycerophosphoinositols are considered to be signalling molecules.

Important biological changes were identified in the metabolomic profiles of women who suffered a subsequent miscarriage compared to those who had a live birth following the endometrial biopsy. The most relevant metabolite classes were triacylglycerides (fatty acid storage molecules), purine and pyrimidine metabolism and ether glycerophospholipids.
Limitations, reasons for caution: This was a first discovery study to evaluate endometrial receptivity using metabolomics in a relatively small selected population of women who suffered recurrent miscarriage. The findings reported here have not been validated in an independent sample set yet.
Wider implications of the findings: Abnormal metabolomic profiles may explain variations in endometrial receptivity and build up on the evidence to support non-chromosomal causes for higher order miscarriages. Validation of the biological changes identified prior to a subsequent miscarriage or live birth may aid in developing a prediction test for use in clinical practice.
Trial registration number: NCT03442335

O-205 The precise identification of the window of implantation using the molecular tool ER Map® in ART cycles significantly improves clinical outcomes

M. Enciso[1], J. Aizpurua[2], B. Rodriguez-Estrada[1], I. Jurado[1], M. Ferrandez-Rives[1], E. Rodriguez[3], E. Pérez-Larrea[3], A.B. Climent[4], K. Marron[5], J. Kennedy[5], J. Sarasa[1]

[1]iGLS, Research and Development, Alicante, Spain ;
[2]IVF-Spain, Gynaecology, Alicante, Spain ;
[3]IVF Donostia, Gynaecology, San Sebastian, Spain ;
[4]IVF-Spain, Statistics, Alicante, Spain ;
[5]Sims IVF Clinic, IVF Unit, Dublin, Ireland

Study question: Does the identification of the window of implantation using the endometrial receptivity test ER Map® improve ART outcomes?
Summary answer: The use of ER Map® test for endometrial receptivity evaluation and personalised scheduling of embryo transfer significantly improves pregnancy rates.
What is known already: The endometrium reaches a receptive status for embryonic implantation around days 19-21 of the menstrual cycle. During this period, known as the window of implantation (WOI), the endometrium shows a specific gene expression profile suitable for endometrial function evaluation. ER Map® is a molecular diagnostic tool able to accurately predict endometrial receptivity status by analysing the gene expression profile of an endometrial biopsy by high-throughput RT-qPCR. In this study, the experience of the clinical application of ER Map® for WOI identification and personalised scheduling of embryo transfer in subfertile couples is presented in detail.
Study design, size, duration: This is a retrospective study analysing endometrial function and ART outcomes of 2256 patients undergoing endometrial receptivity assessment by ER Map® between March 2016 and September 2019. Results obtained when single embryo transfers were scheduled either on the moment of endometrial receptivity (WOI timeframe) as recommended by ER Map®, or deviating from this recommendation are analysed and compared.
Participants/materials, setting, methods: Patients referred for ER Map® analysis were included in the study. Endometrial biopsy samples obtained in a HRT cycle at P_4+5.5 were ER Map® tested and classified into 'Receptive", 'Pre-receptive" or 'Post-receptive". Based on ER Map® results, a recommendation for embryo transfer was performed. Single blastocyst transfers (sET) were performed in all cases. Clinical outcome measures were: positive β-HCG rate [βR], clinical pregnancy rate [CPR] and pregnancy loss rate [PLR].
Main results and the role of chance: ER Map® analysis of endometrial receptivity status showed that 771 out of 2256 patients (34.2%) studied had a displaced WOI.

Analysis of ART outcomes showed a significantly higher clinical pregnancy rate in sETs scheduled within the WOI predicted by ER Map® compared to transfers that deviated more than 12h from ER Map® recommendation (βR 56.09% vs 41.54%, X^2 test p=0.02; CPR 44.35% vs 23.08%, X^2 Test p <0.001). Pregnancy rates were even lower when the deviation from the WOI identified by ER Map® exceeded 24h (βR 56.09% vs 26.92%, X^2 test p=0.012; CPR 44.35% vs 19.23%; X^2 test p=0.004). The deviation of embryo transfers from the WOI predicted by ER Map® had also an impact on the progression of pregnancy. A significant increase in pregnancy loss (~2-fold) was detected in the group of transfers that deviated from ER Map® recommendation compared to transfers performed within the ER Map® WOI (PLR 41.4% vs 21.05%, X^2 Test p <0.022).

This study provides strong evidence that ER Map® endometrial receptivity evaluation can reliably identify the WOI and improve clinical outcomes. Personalisation of progesterone duration pre-treatment before transfer renders significantly improved ART results, increasing the likelihood of pregnancy and reducing the risk of miscarriage.
Limitations, reasons for caution: ER Map® test can serve as a valuable tool to improve ART results, however, to determine the true extent of any clinical benefits, other types of investigations, such as non-selection studies and randomized controlled trials, will also be necessary.
Wider implications of the findings: The application of ER Map® for the identification of cases of WOI displacement and personalised embryo transfer scheduling is an effective strategy for improving ART outcomes. Not only patients suffering from implantation failure but also couples experiencing recurrent miscarriage can benefit from the accurate identification of the WOI.
Trial registration number: Not applicable

O-206 Recurrent pregnancy loss: diagnostic workup after two or three pregnancy losses? A systematic review of the literature and meta-analysis.

M. Van Dijk[1], A. Kolte[2], J. Limpens[3], E. Kirk[4], S. Quenby[5], M. Van Wely[1], M. Goddijn[1]

[1]Amsterdam UMC- University of Amsterdam, Center for Reproductive Medicine, Amsterdam, The Netherlands ;
[2]Recurrent Pregnancy Loss Unit- Fertility Clinic 4071- University Hospital Copenhagen-Rigshospitalet-, Recurrent Pregnancy Loss Unit, Copenhagen, Denmark ;
[3]Amsterdam UMC- University of Amsterdam, Medical library, Amsterdam, The Netherlands ;
[4]Royal Free Hospital, Obstetrics and Gynaecology, London, United Kingdom ;
[5]University Hospital Coventry- Warwick Medical School., Division of Reproductive Health, Warwick, United Kingdom

Study question: Do abnormal test results for definite or probable risk factors for RPL occur with equal frequency in women with two versus women with three or more pregnancy losses?

Summary answer: No difference in prevalence in uterine abnormalities and APS was found, a difference in prevalence of chromosomal abnormalities, thrombophilia and thyroid disorders cannot be excluded.

What is known already: Recurrent pregnancy loss (RPL) occurs in 1 – 3% of all couples trying to conceive. No consensus exists when to perform testing for risk factors in couples with RPL. Some guidelines recommend testing if a patient has had two pregnancy losses whereas others advise to test after three losses. Before trying to conceive, couples and clinicians attempt to find an explanation for their pregnancy losses and a treatment that will prevent a recurrence, especially in cases with modifiable risk factors, such as thyroid disorders and APS. Is it necessary to wait before the next pregnancy loss will occur?

Study design, size, duration: A systematic review of literature and meta-analysis was performed.

Participants/materials, setting, methods: Relevant studies were identified by a systematic search in OVID Medline and EMBASE from inception. A search for recurrent pregnancy loss was combined with a broad search for terms indicative of number of pregnancy losses, screening/testing for pregnancy loss or the prevalence of known risk factors. Meta-analyses were performed in case of adequate clinical and statistical homogeneity. The quality of the studies was assessed using the Newcastle-Ottawa scale.

Main results and the role of chance: From a total of 1985 identified publications, 21 were included in this systematic review and 19 were suitable for meta-analyses. For uterine abnormalities (7 studies, OR 1.00, 95%CI 0.79 – 1.27, I^2=0%) and for antiphospholipid syndrome (3 studies, OR 1.04, 95%CI 0.86 – 1.15, I^2=0%) we found low quality evidence for a lack of a difference in prevalence of abnormal test results between couples with two versus three or more pregnancy losses. We found insufficient evidence of a difference in prevalence of abnormal test results between couples with two versus three or more pregnancy losses for chromosomal abnormalities (10 studies, OR 0.78, 95%CI 0.55 – 1.10), inherited thrombophilia (5 studies) and thyroid disorders (2 studies, OR 0.52, 95%CI: 0.06 – 4.56).

Limitations, reasons for caution: A methodological limitation of this study is the definition of the study groups. On average, 15-20% of women with two losses will experience a loss in the next pregnancy some of the women, some will be in the other group if evaluated at a different time point.

Wider implications of the findings: The results of this study may support investigations after two pregnancy losses in couples with RPL, it should be stressed that additional studies of the prognostic value of investigations used in RPL are urgently needed. An evidenced-based treatment is currently not available in the majority of cases.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 52: AI. A NEW TOOL TO ASSESS ART OUTCOMES AND HELP PATIENTS?

07 July 2020 Parallel 4 17:00 - 18:00

O-207 Patient-oriented counselling: predicting live birth probability (LBP) at each step of the In Vitro Fertilization (IVF) process via machine learning.

V. Grzegorczyk Martin[1], T. Fréour[2], C. Avril[1], A. De Bantel - Finet[1], P. Barrière[2], J.L. Pouly[3], M. Grynberg[4], I. Parneix[5], J. Roset[1], T. Grzegorczyk[6]

[1]Clinique Mathilde, Centre d'Assistance Médicale à la Procréation, rouen, France ; [2]CHU de Nantes, Service de Biologie et Médecine de la Reproduction, Nantes, France ; [3]CHU Clermont Ferrand, Unité de Fécondation In Vitro Département de Gynécologie Obstétrique et Reproduction Humaine Polyclinique de l' Hotel-Dieu, Clermont Ferrand, France ; [4]Hôpital Antoine-Béclère, Médecine de la reproduction et préservation de la fertilité, Clamart, France ; [5]Polyclinique Jean Villar, Centre d'AMP - IFREARES Bordeaux, Bruges, France ; [6]Teranalytics, Data management, Boston, U.S.A.

Study question: How does a machine learning tool for live birth prediction accuracy evolve to counsel couples as demographic, ovarian stimulation, laboratory and transfer data become available?

Summary answer: The weight of individual parameters is not static but evolves at each step of IVF process, contributing to higher accuracies in live birth prediction (C-index).

What is known already: Several predictive models have been developed to help ART clinicians in counselling patients and improve decision-making process. The first validated models, proposed by Templeton et al. in 1996, then improved by Nelson et al. in 2011, integrated demographic and clinical parameters before starting IVF. In 2016, Mc Lernon et al. proposed a new predictive tool based on additional information collected during the first IVF attempt providing an individualized estimate of a couple's cumulative chances of live birth.

To our knowledge, no predictive model exists that dynamically adjusts the LBP throughout the IVF process, as new data become available.

Study design, size, duration: We designed our study on retrospective data of 9587 IVF cycles between 2016-2017 from four IVF centers. Only cycles with verified, consistent and complete data up to final outcome were included.

Parameters were split into four categories following the IVF process: (1) demographic (patient baseline characteristics), (2) ovarian, (3) laboratory and (4) transfer data.

Participants/materials, setting, methods: We implemented a univariate and multivariate evolutive logistic regression, validated with Random Forest, with iterative parameter selection based on 5% significance level. No parameters were included or excluded by default. Validation was internally based on a 70%-30% split. Results are reported as odd ratios and C-index.

LBP were re-calculated by fitting a new model at the addition of each of the four categories, finally yielding probabilities for each fresh or frozen embryo transfer.

Main results and the role of chance: Accuracy of the model in predicting live birth progressively increased from 0.64 to 0.72 as ovarian stimulation, laboratory and embryo transfer parameters were respectively added.

Female age remains strongly predictive of LBP at all steps. After fresh embryo transfers, LBP decreases linearly with increasing female age, whereas its impact becomes significant beyond 37 years for frozen transfers (OR=0.59; 95%CI=0.4-0.85).

Female BMI influence is constant throughout all steps (OR=0.98; 95%CI=0.97-0.99).

Among infertility causes, endometriosis showed the worst impact on LBP.

AFC and AMH are significant predictors when only baseline characteristics are included in the model, but their impact is not statistically significant anymore when the number of retrieved oocytes becomes available.

High gonadotrophin dose negatively impacts LBP in fresh embryo transfers (OR=0.52; CI=0.42-0.64).

Endometrial thickness >7mm positively impacts LBP at the transfer step (OR=1.07; CI=1.04-1.10).

As an example, a 38 year-old woman whose baseline characteristics give her 22% LBP for her first fresh transfer if AMH is 2.5ng/mL, has 22% recalculated chances if 3 oocytes are retrieved and 28% if 8 are collected. If that same woman has an AMH of 1.5ng/ml, her LBP drops to 17%, but increases to 21% and 27% if 3 and 8 oocytes are retrieved, respectively.

Limitations, reasons for caution: Our models need to be further evaluated by performing an external validation: infertile population in France might be slightly different than in other countries (ie: BMI, ethnicity, etc). The limited number of cycles results from deliberate stringent quality control process, whereby only verified, consistent, and complete cycle data were kept.

Wider implications of the findings: The prospect of this work is to develop a practical and evolutive tool to help clinicians accompany their patients by always providing the latest personalized estimates of LBP, before and throughout the IVF intervention, at each fresh and/or frozen embryo transfer.

Trial registration number: not-applicable

O-208 Is IVF making more male embryos and does maternal AMH play a role in this?

K. Hammer[1], C. Cherston[1], S. Vagios[1], C. Sacha[1], D. Pepin[2], C. Bormann[1], M. Kumar Kanakasabapathy[3], P. Thirumalaraju[4], H. Shafiee[5], M. Morris-Sabatini[1]

[1]Massachusetts General Hospital, OBGYN-Reproductive Endocrinology & Infertility, Boston, U.S.A. ; [2]Massachusetts General Hospital, Pediatric Surgical Research Laboratories-Department of Surgery, Boston, U.S.A. ; [3]Harvard Medical School- Brigham and Women's Hospital, Department of Medicine, Boston, U.S.A. ;

[4]Harvard Medical School, Harvard-MIT Division of Health Sciences and Technology, Boston, U.S.A. ;
[5]Harvard Medical School, Department of Medicine, Boston, U.S.A.

Study question: Does anti-mullerian hormone (AMH) predict neonatal sex if undergoing in vitro fertilization?
Summary answer: Increasing AMH levels correlate with an increased trend for male births, likely related to rate of developmental progression of male embryos by day 5.
What is known already: A sex skew toward male neonates after IVF has been described, but it is unclear why this skew exists. This phenomenon may be related to speed at which a male embryo develops to the blastocyst stage leading to an increase in their selection for transfer. We propose that maternal AMH also plays a role in early embryonic development. AMH is known for its role in sexual differentiation and as a marker for ovarian reserve. AMH receptor has been heavily identified in male placentae and fetal membranes and likely impacts early embryonic development.
Study design, size, duration: Retrospective data analysis of 658 IVF cycles from January 2015 to December 2019 at an academic institution in the United States. Data include SART primary outcomes, PGT outcomes, artificial intelligence (AI) data on embryo selection and sex by either PGT or neonatal outcome. We hypothesize that a higher AMH level will lead to more male high-quality embryos on day 5 of embryo culture.
Participants/materials, setting, methods: SART data included women ages 27-44 with AMH levels drawn within 1 year of IVF. PGT cycles additionally included EmbryoScope data using an AI algorithm trained to select the highest quality embryo by morphology for transfer on day 3 and day 5 of development. Results were analyzed by AMH level within quartiles for the following endpoints: ratio male:female neonate sex, ratio male:female PGT result, sex of day 3 and day 5 embryo selected using AI.
Main results and the role of chance: SART contained 658 singleton newborns, 53.8% (n=354) were male and 46.2% (n=304) female. AMH correlated with male neonate; for every 1ng/mL increase in AMH the odds for a male neonate increased 5.4%, p=0.017. Quartile analysis demonstrated that at or above the seventy-fifth percentile, AMH=5.11ng/mL, was associated with male sex; male to female 60.7% (n=99) vs 39.3% (n=64). AMH less than the seventy-fifth percentile had a male to female ratio 51.5% (n=255) vs 48.5% (n=240), p=0.04.

PGT-A cycles, n=209, had more female than male embryos, 52,1% (368/706 embryos) female vs 47.9% (368/706 embryos) male. AI analysis of the highest quality embryo demonstrated a difference in sex ratio between day 3 and day 5. Day 3 cohort favored female embryos at higher AMH levels than male embryos, female (median 2.3, IQR 1.4-3.8) vs male (median 1.3-3.0), p=0.042. Day 3 embryos and AMH above the seventy-fifth percentile demonstrated a trend towards female sex, male 35.7% (n=10) vs female 64.3% (n=18), p=0.136. Day 5 embryos had comparable AMH values for both sexes, male median 2.1 (IQR 1.4-3.7) and female median 2.1 (IQR 1.3-2.9), p=0.45. AMH above the seventy-fifth percentile had an increased trend toward male embryo selection 67.9% (n=19) male vs 32.1%(n=9) female, p=0.23.
Limitations, reasons for caution: This is a retrospective review of SART data combined with data from a verified artificial intelligence algorithm to identify the highest quality embryos during development. More work is needed to elucidate how AMH exposure of the oocyte may differently impact the development of male vs female embryos.
Wider implications of the findings: AMH levels in the follicle may have implications for early embryo development kinetics. Providers must account for sex differences in embryo development so as not to artificially skew the sex ratio when selecting embryos for transfer.
Trial registration number: not applicable

O-209 Time to pregnancy for women using a fertility awareness based mobile application to plan a pregnancy

J. Pearson[1], S. Rowland[1], C. Favaro[1], E. Berglund-Scherwitzl[1], R. Scherwitzl[1], K. Gemzell-Danielsson[2], J. Harper[3]

[1]Natural Cycles Nordic AB, Luntmakagatan 26, Stockholm, Sweden ;
[2]Karolinska Institute, Head Department of Women´s and Children´s Health, Stockholm, Sweden ;
[3]UCL Institute for Women's Health, Department of Reproductive Health, London, United Kingdom

Study question: What is the time to pregnancy for users of the Natural Cycles fertility awareness based mobile application to identify the fertile window?
Summary answer: Women <35 years, with no anovulation, regular cycles and who regularly logged sex (52% of cohort) had a 12-month pregnancy probability of 96%.
What is known already: Women trying to get pregnant are encouraged to identify their fertile window using methods such as their menstrual cycle dates, basal body temperature, changes in cervical mucus and lutenising hormone surge identification. But these methods can become time consuming and stressful. Using an app which can calculate the fertile window using an algorithm may be easier and more accurate. This is one of the first studies to examine the time to pregnancy for women using an app that identifies the fertile window.
Study design, size, duration: This observational study included 5376 women with an age range of 18 to 45 years. They used the app in Plan pregnancy mode between September 31st 2017 and August 31st 2018 to allow at least 12 months of potential usage up to the cutoff date of September 1st 2019.
Participants/materials, setting, methods: The time to pregnancy was analysed related to age, gravidity, BMI, cycle length variation, average cycle length, and behavioural characteristics such as frequency of sexual intercourse via hazard ratios (HRs) using Cox regression adjusted for fixed covariates.
Main results and the role of chance: 3166 women achieved pregnancy within 13 cycles, while 570 women were still trying to get pregnant at the end of the observation period. Overall the one-year pregnancy probability was 83% (95% CI: 82%-84%). The mean time to pregnancy for the whole cohort was 3.3 cycles (95% CI: 3.2-3.4). Women <35 years, with no anovulation, regular cycles and regularly log sex (52% of cohort) had a 12 month pregnancy probability of 96% and a mean time to pregnancy of 2.4 cycles (95% CI: 2.2-2.6). Lower pregnancy probability was associated with the following exposures: ages 35 to 45 (HR 0.69, 95% CI: 0.61-0.78), high cycle length variation (HR 0.89, 95% CI: 0.77-1.01) and sexual intercourse logged on less than 10% of days (HR 0.84, 95% CI: 0.71-1.01). Anovulatory cycles were also associated with a worse outcome with a one-year pregnancy probability of 72%.
Limitations, reasons for caution: Users had higher educational level and lower BMI than would be expected in the general population. Close to 50% had characteristics associated with sub-fertility such as age > 35 years, highly variable cycle length or anovulatory cycles. Sex logging data was incomplete which limited the conclusions that could be made.
Wider implications of the findings: Fertility awareness apps have an important role in educating women and their partners about fertility and facilitating discussions around the topic. In the future smart algorithms may facilitate early identification of couples who may benefit from infertility assessment.
Trial registration number: 2016/2037-31

O-210 Interactive information provision during IVF/ICSI treatment by using an app: A randomized controlled trial

M. Keijsers[1], T. Timmers[2], I. Van Rooij[1], J.M.J. Smeenk[1]

[1]Elisabeth Twee Steden Hospital, Obstetrics & Gynecology, Tilburg, The Netherlands ;
[2]Interactive Studios, software development, Rosmalen, The Netherlands

Study question: An application (app) has been developed providing patients with information in an interactive way; will the use of this tool result in a change of knowledge, self-reliance and care consumption?
Summary answer: Patients in the app group achieved a higher level of knowledge and were more satisfied with treatment compared to the control group.
What is known already: Patients undergoing an ivf/icsi treatment experience a lack of knowledge, which causes (unnecessary) stress during their treatment. Furthermore, they receive a lot of information in a limited timeframe in the clinic often resulting in an insufficient uptake of relevant details. Patients would like to see an improvement in providing information. Routinely providing comprehensible and structured information, in particular about the practical aspect of the treatment, in a patient controlled way could be a solution.
Study design, size, duration: A single center randomized controlled trial was conducted. The app group had access to the informative and personal app,

including informative videos and quiz questions.The control group received the current/normal form of information provision. Each patient received four questionnaires at fixed timepoints prior to, during and after treatment. The questonnaires focussed respectively on information provision, self-reliance, care consumption, level of knowledge and finally the satisfaction of care during treatment was evaluated.

Participants/materials, setting, methods: In the period from April 2018 to September 2019, 51 patients were included into the study. Native patients, so without any experience with the use of gonadotrophins and with a medical indication for IVF/ICSI treatment were invited to participate in a randomized controlled trial prior to their intake regarding the IVF/ICSI treatment. Twenty-two patients were included in the control group and twenty-nine patients in the app group.

Main results and the role of chance: The groups were found not to differ significantly on baseline characteristics and outcome parameters. The patients in the app group scored higher on the QPP-IVF questionnaire (indicating satisfaction) than the patients in the control group, although this did not reach significancy (52,89 versus 47,92, p=0,13). Two days after the ivf intake in the clinic both the control group (from 17,33 to 23,25, p=0,22) and the app group (from 20,74 to 26,58, p=0,02) scored higher in the relative level of knowledge compared to baseline findings. Only the increase in the app group was found to be significant. Five days after the ovum pick up, a significant increase in the level of perceived knowledge is also observed in the app group (from 20,74 to 26,89, p=0,05), but not in the control group (from 17,33 to 26,00, p=0,48). No significant differences were found on self-reliance and care consumption (p>0.05).

Limitations, reasons for caution: The limited sample size of the pilot study is a reason for caution. The observed differences need to be replicated in a larger study and in a multicentred design.

Wider implications of the findings: Knowledge on the use of a patient journey app is limited within IVF/ICSI. The preliminary results indicate that the app could help patients in the uptake of information at their own pace, resulting in more knowledge and satisfaction. This could also have a beneficial effect on care consumption and costs.

Trial registration number: NA

SELECTED ORAL COMMUNICATIONS
SESSION 53: CONTROVERSIES IN ART
07 July 2020 Parallel 5 17:00 - 18:00

O-211 Commercial influences on assisted reproductive technology: a scoping review

W. Lipworth[1], M. Wiersma[1], N. Ghinea[1], A. Newson[1], I. Kerridge[1], C. Waldby[2], W. Ledger[3], R. Norman[4]

[1]University of Sydney, Sydney Health Ethics, University of Sydney, Australia ; [2]Australian National University, Research School of Social Sciences, Canberra, Australia ; [3]University of New South Wales, Obstetrics & Gynaecology, Sydney, Australia ; [4]University of Adelaide, Robinson Research Institute, Adelaide, Australia

Study question: How are commercial influences on assisted reproductive technology (ART) understood, debated and studied in the academic literature?

Summary answer: Commercial influences raise a numerous ethical, organisational, economic and clinical issues. These are discussed in diverse contexts, not limited to direct discussions of commercialisation

What is known already: While advances in technologies have increased the range of ART options available to patients, concerns exist about the steps taken by ART providers to ensure that their businesses are successful, profitable and sustainable (henceforth "commercial influences" on ART). Distinguishing the key issues associated with these commercial influences is complicated by the diversity of ways and contexts in which they are discussed, and there have been no systematic attempts to draw these together.

Study design, size, duration: This scoping review used the methodological framework first proposed by Arksey and O'Malley, and later refined by Levac and Peters. It involved five key stages: 1) identifying the research question; 2) identifying relevant studies; 3) study selection; 4) charting the data; and 5) collating, summarising and reporting results.

Participants/materials, setting, methods: Articles were included if they addressed one or more of the following topics: 1. What are the factors that shape the commercial organisation of ART? 2. How are commercial influences currently identified, appraised, regulated and managed? 3. How should commercial influences be addressed, by whom, and with what guidance? The search strategy was developed based on keywords and index terms related to commercialisation of ART in PubMed based on Medical Subject Headings (MeSH) terms.

Main results and the role of chance: Commercial influences on ART are a frequent topic of discussion and critique in the academic literature, however these discussions are widely dispersed and often implicit in discussions of 1. The pricing of ART services, and variability in these costs 2. The number and timing of interventions, particularly when it comes to the use of more invasive (and expensive) interventions in patients who might benefit from less invasive treatments and patients in whom success is unlikely 3. The expansion into non evidence-based interventions 4. The ways in which interventions are marketed and justified to patients 5. The workings of international markets and 6. Other commercial interests, such as joint ownership of diagnostic, counselling and hospital services and relationships with other health-related industries.

Limitations, reasons for caution: A scoping review is a semi-systematic literature review that aims for thematic saturation rather than aiming to collect the full range of articles on a topic. In cannot, therefore, draw quantitative conclusions about the literature. Our search was also limited to articles published in English.

Wider implications of the findings: The implications of the commercialisation of ART cannot be understood without systematically considering a wide range of ethical, organisational, political, economic, scientific and clinical issues, across a diverse range of literatures. We provide a novel "map" of these issues that can help to guide debate on this highly contentious topic.

Trial registration number: Not applicable

O-212 Expanded carrier screening in medically assisted reproduction: An ethical exploration of a new screening option in the light of professional and parental responsibilities

S. Van der Hout[1], W. Dondorp[1], G. De Wert[1]

[1]Maastricht University, Health- Ethics & Society, Maastricht, The Netherlands

Study question: May professionals recommend that applicants of assisted reproduction undergo expanded carrier screening, and that identified carrier couples apply for pre-implantation genetic testing to avoid the birth of an affected child?

Summary answer: While an offer of ECS should be non-directive, carrier couples of very serious disorders should have access to MAR only if they apply for PGT.

What is known already: Expanded carrier screening (ECS) entails a screening offer for multiple recessive disorders simultaneously, and allows testing of couples regardless of ancestry. A growing number of European fertility clinics offer ECS on a routine basis. However, little attention has been given to the ethics of ECS in the context of medically assisted reproduction (MAR). MAR-professionals have indicated a need for ethical guidance, as an offer of ECS addressing MAR-patients raises ethical questions that do not arise in other carrier screening contexts: the involvement of MAR-professionals in creating the child implies that both professional and parental responsibilities should be taken into account.

Study design, size, duration: In this ethics study we explore the morally relevant aspects of the scenario of offering ECS to MAR-patients, focusing on possible tensions between considerations of reproductive autonomy and parental/professional responsibility. This study is part of a larger project in which we will use the findings to conduct an exploration of relevant stakeholder views. The overall aim is to contribute to a robust normative framework that can guide the practice of ECS in MAR.

Participants/materials, setting, methods: This is a desk research study bringing together the scientific literature relevant to the possible (wider) introduction of ECS in MAR and debates conducted in philosophical and applied ethics literature on the nature and scope of relevant principles including

'reproductive autonomy', 'professional responsibility' and 'responsible parenthood'. We use the method of 'wide reflective equilibrium' as a framework for our analysis. This method has a proven value for the normative analysis of bioethical problems.

Main results and the role of chance: MAR-professionals have a responsibility to take account of the welfare of the child that they are causally and intentionally involved in creating. ESHRE has recommended that they should refrain from providing assistance when there is a high risk that the child will have a seriously diminished quality of life. What follows from this norm with regard to offering ECS to MAR-patients? May (or should) professionals recommend, or even insist, that patients have ECS, and make use of pre-implantation genetic testing (PGT) or other preventive options when both partners are identified as carriers? We recommend that this depends on a) the level of genetic risk; and b) the severity of the disease under consideration. If MAR-patients have an a priori low carrier risk, ECS should be offered in a non-directive way and facilitate autonomous reproductive decision-making. However, if both partners are identified as carriers of a serious genetic disorder, professionals may well be justified in urging them to actively consider PGT, or even make access to MAR conditional on the patients' use of preventive options. We conclude that the preventive options created by new genomic testing possibilities are not morally indifferent, but may bring along new professional and parental responsibilities.

Limitations, reasons for caution: This is an ethical exploration aimed at contributing to further debate about the responsible introduction of ECS in MAR.

Wider implications of the findings: Our exploration of the relationship between respect for autonomy and acknowledging professional and parental responsibilities for the welfare of the future child has a wider relevance for the ethics of (shared) decision-making in the highly dynamic field of MAR as a whole.

Trial registration number: Not applicable

O-213 Ethical justifications of Spanish Fertility Society to preserve anonymity of gamete donation

R. Nunez - Calonge[1], M. Muñoz[2], F. Abellan[3], I. Cuevas[4], L. Feito[5], A. De la Fuente[6], L. Martínez[7], D. Mataro[8], M. Roca[9]

[1]URA, Ethics and Reproduction, Madrid, Spain ;
[2]IVIRMA, Medical Director, Alicante, Spain ;
[3]Sociedad Española de Fertilidad, Legal, Madrid, Spain ;
[4]Hospital General, IVF Laboratory, Valencia, Spain ;
[5]Faculty of Medicine- Complutense University, Public Health & Maternal and Child Health, Madrid, Spain ;
[6]Instituto Europeo de Fertilidad, Medical Director, Madrid, Spain ;
[7]Hospital Virgen de las Nieves, Reproduction, Granada, Spain ;
[8]Eugin Clinic, Reproductive Endocrinology and Infertility, Barcelona, Spain ;
[9]Barcelona University, Psychology, Barcelona, Spain

Study question: What is the view of Spanish Society of Fertility (SEF) regarding ethical aspects of the removal of anonymity in gamete donation?

Summary answer: SEF recommends disclosure to the donor-conceived children their biological origin preserving the anonymity since there are no convincing ethical arguments for removal.

What is known already: Although most gamete donations in the world are made anonymously, a growing number of countries have questioned the morality of preserving anonymity, and laws are being enacted that allow children born by donation to identify donors.

The main criticisms of the anonymity of gamete donation focus on the primary interest of donor-conceived children, and more specifically on health and interest in forming their own identity. On the other hand, those who support anonymity argue that its practice respects the interests of the donor as well as the parents' wishes to determine the best interest of offspring.

Study design, size, duration: Reflecting concern about the anonymity of gamete donation, the Spanish Society of Fertility convened a focus group with key figures in ethics, law and reproductive medicine to identify scientific, psychological, legal and ethical arguments supporting the anonymous gamete donations. The project was set-up as a qualitative study to call in to question the justifications that are often thought to ground a right to know one's genetic origins and took place between May and October 2019.

Participants/materials, setting, methods: One of the objectives of the focus group was to deliberate about the ethical justifications that support the right to know the genetic origins, mainly based on the protection of health and the right to identity, but also taking into account other values that affect the different participants in the donation For this, we used the deliberative method to deal with conflicts, as it seeks to protect the values that may be at stake.

Main results and the role of chance: Spanish Society of Fertility has produced an Anonymity in Donations Framework Document, divided into five distinct parts: demographic, clinical, psychological, social, ethical and legal aspects. From an ethical perspective, the following arguments regarding gamete donation anonymity were suggested:

Health of children: Spanish policy, despite its requirement of anonymity, explicitly indicates that donor-conceived individuals should have access to non-identifiable information about the donor, including medical information, so, the anonymous donation is not incompatible with providing relevant medical information.

Sense of identity: there is no solid empirical evidence to show that children conceived by the donor in general suffer "genealogical confusion".

Other values examined were: autonomy of the parents and the right to privacy, genetic inheritance, donor confidentiality, justice, responsibility, quality and economic value.

Following the bioethical deliberation mentioned above, and after having examined the main values that become part of the two extreme courses (anonymity of the donation against its suppression), the best intermediate course would be the one that tried to safeguard the greater number of values involved, namely, donor-conceived individuals are morally entitled to access general (non-identifying) information about their origin.

Limitations, reasons for caution: Although there is no evidence of any conflict in Spain for the rule of anonymity, the opinion of the patients, donors and offspring, would be lacking.

Wider implications of the findings: Before introducing mandatory disclosure of the donor´s identity, the commitment should be to ensure that this change entails advantages for those involved, which have not been demonstrated so far. Strategies should be adopted to implement education programs that do not stigmatize the fact of being born through donor gametes.

Trial registration number: Not applicable

O-214 First sperm and egg bank in Latin America with open identity donors: a five-year study on patients' and donors' preferences between anonymous and open-identity donation.

P. Regalado[1], V. Rawe[1]

[1]REPROBANK, Research, Buenos Aires, Argentina

Study question: Would gamete donors and patients choose open-identity given the chance? Several countries worldwide have laws regulating anonymity in gamete donation, mostly European and North American with opposing standpoints between them.

Summary answer: Ours results show many patients demanding open identity and half of gamete donors willingly donating this way, concurring with international tendency to unveiling donor identity.

What is known already: In Latin America few countries have laws regulating Assisted Reproductive Techniques (ART) and anonymity donation is set by default. The lack of empirical data is alarming: having non-official statistics, national surveys or publications addressing open identity vs anonymous donation. A tendency toward unveiling donor identity to offspring is increasing in Europe and North America. However, in Latin America few countries have laws regulating ART and anonymity donation is set by default. Our study is rich and pioneer for Latin American, it shows our cultures demand to allow access to genetic origins by lifting anonymity and leave the choice to offspring.

Study design, size, duration: Report of 5 years of experience of the first open identity program (PIA) in Argentina, offering both anonymous and open identity donor sperm samples and later extended to eggs. We intended to discover donors' and patients' choice on open vs anonymous donation, reasons and motivations for choosing either. We gathered information from all donors and patients that participated or went through ART.

Participants/materials, setting, methods: The sample includes 192 donors (132 sperm and 60 egg), 2391 ART patients and over 150 parents of open identity donors´ offspring. Descriptive, correlational and inferential statistics were

applied with parametrical and non-parametrical analysis. Also, we surveyed the sample with two *ad hoc* questionnaires regarding reasons or setbacks for choosing open or anonymous donations, opinions on current legislation on the matter, future plans on identity revelation to offspring, among other queries.

Main results and the role of chance: Statistics show that open identity donation was chosen by 55% of egg donors, 48.5% of sperm donors and 29% of ART patients. Regarding donors, more egg than sperm donors chose open identity, however the difference was not statistically significant. Regarding patients, results showed statistically significant differences based on type of family structure: open identity donation is chosen in 1 out of 10 heterosexual couples, 3 out of 10 lesbian couples, and 4 out of 10 single women. More in depth results are discussed in relation to patients' and donors' general reasons, attitudes and motivations toward open identity and anonymity. Also, post-treatment parents with open identity offspring past choice satisfaction is addressed.

An international wide controversy has existed since sperm donation became a part of ART. Anonymity continues to depend on judicial regulations. Empirical research needs to guide laws based on ethical and social-need grounds. The past decade has shown an international tendency towards removing anonymity or allowing access to genetic origins. Anonymity in many countries continues to be defended while social movements and organizations of donor offspring continue to demand the human right to access genetic origins. Research on open identity vs. anonymity mostly reflects European and North American cultures.

Limitations, reasons for caution: Samples were gathered from a single gamete bank because it is the only one in the country offering an open identity program. Samples don't represent argentine ART patients. However, it does show a tendency in favor of what has been found in other countries.

Wider implications of the findings: Our findings are rich and pioneer for Latin American, evidencing a cultural demand to address anonymity. Few countries in Latin America have ART laws. Research is fundamental for future legislations.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 54: MODERN TECHNIQUES PROMOTE VARIETY IN FERTILITY NURSING RESEARCH

07 July 2020 Parallel 6 17:00 - 18:00

O-215 The Effect of Hypnofertility on Cortisol Levels, Fertility Preparedness and Pregnancy Rates in Women Undergoing InVitroFertilization: A Randomized Controlled Study

S. Fata[1], M. Aluş Tokat[1]

[1]Dokuz Eylul University, Obstetric and Gynecological Nursing, Izmir, Turkey

Study question: Does Hypnofertility have remarkable effect on cortisol levels, fertility preparedness and pregnancy rates?

Summary answer: Hypnofertility is a powerful and effective mind-body program and aims to improve relaxatation, regulate hormone secretion and increase the pregnancy rates.

What is known already: It is found that there is a negatively correlation between stress levels and fertilization rates, pregnancy rates, total births, live births. There is information in the literature that controling of cortisol levels is important and can give idea about stress experienced before starting assisted reproductive treatment. Health professionals can be enterprise some applications to reduce the stress and cortisol levels. One of the methods that can reduce stress is Hypnofertility. Hypnofertility is a powerful and effective mind-body program with aim to relax couples, improve the preparedness to treatment process and therewithall increase the pregnancy rates.

Study design, size, duration: It was randomized controlled trial conducted in Dokuz Eylül University InVitroFertilization (IVF) Center in Turkey between December 2017-March 2019. The 61 women with unexplained infertility (30 women for intervention group and 31 women for control group) were reached. The women were applied intervention or control procedure in four face-to-face session (first treatment day, second control day, OPU, embryo transfer) and one mobile session (from embryo transfer day to pregnancy test day) throughout IVF treatment cycle.

Participants/materials, setting, methods: Interventions based on Hypnofertility including affirmations, visualization, imagination and relaxation were applied to women undergoing with IVF treatment from the first day of treatment to the day of pregnancy test. The standart IVF center follow up process was provided to control group. The cortisol level was evaluated trough saliva sample (four times), for determining preparedness of women the Fertility Preparedness Scale (three times) was applied and pregnancy result was evaluated by blood beta HCG test.

Main results and the role of chance: The group equivalence on baseline socio-demographic and infertility characteristics was shown in similar (X^2: 0.007, p: 0.06; X^2: 4.419, p: 0.93). There was no significant difference between the two groups in terms of fertility preparedness evaluated at baseline (t=0.930, p=.35). Although there was higher improvement in fertility preparedness in intervention group (second session p=0.04, third session p=0.01), the results of repeated measures, showed no difference in total in terms of group*time (F=2.177, p=.13). Saliva samples collection time of both groups were similar (p=.84; p=.41; p=.68; p=.50, respectively). When the cortisol levels of the women in the intervention and control groups were evaluated, a statistically significant difference was found in terms of group*time interaction (F=4.187, p=.00). It was observed that the interventions decreased the cortisol levels of the women in the intervention group compared to the control group (p=.00). Cortisol levels of the intervention group gradually decreased during the treatment period (p=.01). The last measurement has the lowest results in spite of the most stressful days of treatment (the embryo transfer day). Even though interventions relieved the women during treatment process, there was no the expected effect on pregnancy outcomes (X^2: 0.098, p=.75).

Limitations, reasons for caution: The main limitation of this randomized controlled trial was the limited number of samples. Since the cost of each measurement of cortisol level in saliva measured in the study was high, and the support obtained from University Scientific Research Project Coordination Unit was limited.

Wider implications of the findings: Although interventions based on Hypnofertility not effect pregnancy clinical outcomes, they reduced the cortisol levels of women. Also the fertility preparedness was improved in clinically meaningful level.

Trial registration number: NCT04141436

O-216 Assessment of a French web-based patient decision aid (ptDA) of ertility preservation (FP) for women with breast cancer

A.C. Benoit[1], G. Moutel[2], R. Morello[3], G. Grandazzi[2], A. Mayeur[1], M. Grynberg[1]

[1]Antoine Beclere University Hospital, Reproductive Medicine and Fertility Preservation, Clamart, France ;
[2]Normandie University, Inserm U1086- ANTICIPE, Caen, France ;
[3]University Hospital- Caen, Biostatistics and Clinical Research Unit, Caen, France

Study question: Does a French web-based ptDA in addition to standard counselling by a specialist in reproductive medicine improve informed choice of women with breast cancer?

Summary answer: The web-based ptDA we have developed improves patients' knowledge and informed choice without influencing their attitude towards FP and decrease the level of decisional conflict.

What is known already: Chemotherapy may cause infertility in young survivors of breast cancer, impairing their quality of life in the long term. Various FP techniques increase the likelihood of survivors becoming genetic mothers. The complex emotional situation, the need to make the right decision in a short space of time and conflicting ideas about life and death may mean that the decision about FP is a difficult one to take. Weighing up the risks and benefits of FP options may seem a mammoth task. Use of a ptDA could be increase patients' knowledge of treatment options and congruency between informed values and care.

Study design, size, duration: The study was a single-center randomized controlled trial conducted in the FP department of an academic hospital in France from September 2018 to November 2019. After being referred by oncologic

centers to specialists in reproductive medicine for fertility counselling, women were randomly assigned to the control group with usual care or to the intervention group with use of the web-based ptDA before and during counselling.
Participants/materials, setting, methods: A total of 125 women aged 18 to 40, with a primary diagnosis of breast cancer, who have not yet started any cancer treatment and able to read, write and speak French were included and randomized to the control group (n=65) or the intervention group (n=60). We have compared the decision-making process between control group and intervention group by quantitative data collected about fertility-knowledge, attitudes towards FP, and decisional conflict after the FP consultation.
Main results and the role of chance: Better knowledge was found in the intervention group (8.6/10 (±1.34)) compared to the control group (6.49/10 (±1.89)). In both groups, patients had a positive attitude towards FP (96%). The final decision was not influenced by membership in one group or another. Thus, at the end of the FP consultation, 73.6% (92/125), i.e. 69.2% (45/65) of patients in the control group and 78.3% (47/60) of patients in the intervention group, chose to keep their oocytes, embryos and/or ovarian tissue. The proportion of informed choice was statistically higher in the intervention group than in the control group (respectively 75% versus 38.5%, p<0.001). In addition, the level of decision-making conflict among patients in the intervention group was lower than in the control group (respectively 14 (±12.5) versus 21.9 (±15.7), p<0.01).
Limitations, reasons for caution: Investigators were not be blinded to the intervention, which represents a limitation. Furthermore, the study was specifically focused on women with breast cancer. Therefore, the results are not applicated to other malignancies.
Wider implications of the findings: The present study led to the development of the first French language ptDA of FP. Our results suggest that a French web-based ptDA has the potential to help supporting women with breast cancer to make informed fertility-related decisions and might decrease decisional regrets.
Trial registration number: NCT03591848

O-217 Addressing the psychosocial and informational needs of men with severe male factor infertility (MFI) with a peer-support platform

E. Lowndes Stevenson[1], K. Baker[2], K. McEleny[3]

[1]Duke University Medical Center, School of Nursing, Durham NC, U.S.A. ; [2]Duke University Medical Center, Department of Urology, Durham, U.S.A. ; [3]Newcastle upon Tyne Hospitals NHS Trust, Newcastle Fertility Center, Newcastle-upon-Tyne, United Kingdom

Study question: What do men with MFI desire in order to address their psychosocial and educational needs during the fertility process?
Summary answer: Men indicate desiring digital health communication and anonymous digital platforms to meet their psychosocial and informational needs.
What is known already: Men with MFI experience higher levels of psychological challenges such as depression and sexual dysfunction than fertile men. Men respond differently than their partners to the infertility diagnosis. Our previous research found that men employed avoidance strategies/did not disclose infertility status to social networks, experienced affective symptoms (sadness, shock, disbelief, denial), and lacked access to evidenced-based information in part because of their margination within the fertility process, secondary to the female-facing nature of fertility healthcare.
Study design, size, duration: This IRB-approved prospective qualitative study recruited men seeking a MFI consultation with a urologist between 2018 and 2019. Men over 18 years of age with primary or secondary MFI were phone interviewed after providing informed consent. Each digitally recorded interview lasted between 15 and 40 minutes and was transcribed verbatim. Nineteen participants were recruited (until data saturation reached).
Participants/materials, setting, methods: Inclusion criteria were men (≥ 18 years old) seen for a new patient MFI consultation having received a MFI diagnosis during the preceding 12 months and could read/write English. Exclusion criteria were a history of vasectomy or stated absence of a fertility concern despite a fertility related diagnosis. Nineteen men were recruited from a fertility urology clinic in a large academic medical center. Interviews were conducted by phone. Data were analyzed using content analysis.
Main results and the role of chance: Mean age was 35.3 years. Results indicated that participants prefer technology based communication to receive health information (i.e. 'mychart') because of the ease, access, and efficiency. Participants were not amenable to alternative medical structures (i.e. group sessions for emotional support), but were interested in anonymous digital platforms to meet psychosocial needs. In response to these data, as well as previous study team data, our team developed an internationally-focused and non-commercial peer-support platform (https://all-about-fertility.com/) to allow men with MFI to be able to disclose to others with MFI and seek anonymous emotional support online, whilst also receiving accurate and unbiased medical information to support their fertility journey and decision-making. Contributions from global Reproductive Health/Science experts provides men with confidence when seeking health information.
Limitations, reasons for caution: As the results are based on qualitative data from 19 men with MFI, the results cannot readily be generalized to larger populations. Implementation of the peer-support platform is still being optimized and its usefulness and impact to meet stated goals will be evaluated/reported in the coming years.
Wider implications of the findings: Men with MFI have unmet psychosocial and informational needs. Development of a peer-support platform is a positive step in addressing some of these needs and has the potential to have a wide reach into areas with limited support (geographically, economically).
Trial registration number: n/a

O-218 A preparatory information movie for the first oocyte aspiration in addition to care as usual: protocol for the POAM randomized controlled trial

L. Dias[1], P. De Loecker[2], T. D'Hooghe[1], K. Peeraer[3], E. Dancet[1]

[1]KU Leuven, Regeneration and Development, Leuven, Belgium ; [2]GZA Ziekenhuizen campus Sint-Augustinus, Gynaecologie - Verloskunde - Fertiliteit, Wilrijk, Belgium ; [3]UZ Leuven, Regeneration and development, Leuven, Belgium

Study question: Can a preparatory information movie on oocyte aspiration (POAM) decrease the anxiety of women on the day of their first oocyte aspiration? A protocol for a randomized controlled trial.
Summary answer: This RCT protocol provides a framework to examine the effect of a preparatory information movie on oocyte aspiration on women's anxiety and other secondary outcomes.
What is known already: Limited success rates of the fertility clinic trajectory could be increased by preventing discontinuation, a couple's decision to stop treatment despite financial stability and ongoing wish for a child. The main reason for discontinuation is distress, which increases during an IVF cycle, with anxiety peaks on the days of oocyte aspiration and pregnancy test. Moreover, pre-treatment anxiety predicts discontinuation from especially the first IVF cycle. Detailing procedural steps of out-patient clinic invasive procedures decreases patient's anxiety. Information leaflets however often increase anxiety because they can be very complicated. An educational movie simply showing what will happen can overcome this problem.
Study design, size, duration: In the POAM-RCT 190 women are randomized (1:1 allocation ratio; computerized randomization) during a recruitment period of 19 months to the parallel groups of 'care as usual' (a one-hour face-to-face information session 1-3 months prior to the start of treatment on all procedural steps of an IVF cycle, also summarized on a take-home leaflet) or 'care as usual supplemented with the Preparatory information on Oocyte Aspiration Movie (POAM)'.
Participants/materials, setting, methods: The setting for this monocentric interventional single-masked RCT is the fertility clinic of the GZA Ziekenhuizen, a private hospital in Belgium. Dutch speaking women with Dutch speaking male partners who are about to start their first IVF cycle (with or without ICSI or PGT) are eligible. Women who already experienced an IVF cycle and therefore know the patient journey on the day of the oocyte aspiration are not eligible.
Main results and the role of chance: In case of randomization to the intervention group, couples receive the day prior to their first oocyte aspiration, in addition to 'care as usual', a secured link to the POAM (5-minute movie visualizing the patient journey on the day of oocyte aspiration detailing which procedures will happen when, in which room, in the presence of which persons) by email.

The primary outcome of this RCT is women's state anxiety assessed with the reliable STAI-state questionnaire. Secondary patient-related outcomes are men's

state anxiety and also women's and men's infertility-specific distress on the day of the oocyte aspiration, assessed with the Infertility-Distress Scale. Previous research in the field of reproductive medicine showed that men are significantly less distressed if they receive an information leaflet before a diagnostic sperm test. Finally, women's and men's intervention evaluation are assessed by questioning how often they watched the movie and whether or not they would recommend it.

Secondary clinical outcomes that will be followed-up in the electronic medical records are clinical pregnancy, miscarriage, IVF delay, IVF discontinuation and cumulative pregnancy. The evidence on whether women's anxiety affects (cumulative) clinical pregnancy rates and miscarriage rates is conflicting.

Limitations, reasons for caution: A potential limitation of this RCT is selection bias, as women who are more anxious or stressed will be less likely to participate. Randomization aims to adjust for this effect. Another potential limitation is the single-masked character of the RCT as only the statistician will be masked during data processing.

Wider implications of the findings: With this RCT, we expect to demonstrate the value of additional information using a preparatory movie in couples undergoing their first oocyte aspiration. If these couples are positively affected, this technique should be implemented in each fertility clinic. We also want to show the value of publishing a study protocol.

Trial registration number: NCT03717805

human reproduction

ESHRE 2020 / Oral presentations

INVITED SESSION

SESSION 55: COCHRANE SESSION - BETTER EVIDENCE, BETTER POLICIES

08 July 2020 Parallel 1 08:30 - 09:30

O-219 Better RCTs in reproductive medicine

M.Van Wely[1]

[1]*Academic Medical Center, Center for Reproductive, Amsterdam, The Netherlands*

O-220 Where do we go after the RCTs

S. Cheshire[1], **P. Thompson**[2], **C. Ettinghausen**[3], **D. Halai**[4]

[1]*HFEA, Chair of the Board, London, United Kingdom*
[2]*HFEA, Chief Executive, London, United Kingdom*
[3]*HFEA, Director of Strategy and Corporate Affairs, London, United Kingdom*
[4]*HFEA, Head of Scientific Policy, London, United Kingdom*

Abstract text

ART is a fast moving and innovative area of medicine. And while innovation can be a force for good, offering patients improved chances of success, too often new drugs, treatments or equipment are introduced with little robust evidence that they will improve outcome. For regulators this presents a difficulty: how best to encourage innovation while protecting the patient?

More high-quality research including Randomised Controlled Trials, meta-analyses, and follow-up of patients is needed. But where do we go if these are not readily available now?

The UK's Human Fertilisation and Embryology Authority (HFEA) is ~30 years old and has led the way in regulation in this sector. Our experience of licensing mitochondrial donation suggested a different way of thinking about regulation and innovation, where good regulation provides the conditions that encourage innovation while ensuring every patient has access to the right information, at the right time.

We in the fertility sector in the UK are concerned that many patients are being frequently offered, and charged for, optional extras to their treatment which claim to improve their chances of having a healthy baby. These additional therapies and techniques are collectively known as treatment 'add-ons'. They cover a range of interventions including genetic tests, drugs, surgery and equipment. The problems posed by treatment add-ons are well known but crucially, the evidence base for most add-ons is not robust. Over recent years, there has been a significant increase the number of clinics, both in the UK and elsewhere, offering additional treatments – in 2019, 77% [DH1] of clinics in England offered at least one add-on according to their websites.

This talk will focus on the actions the HFEA is taking to encourage more responsible innovation in the way in which clinics offer treatment add-ons, and the way in which patients can understand the risks and benefits involved. The ultimate aim has always been to balance scientific and medical advancement with individual rights and needs. Our add-ons work touches on the quality of evidence, patient information and informed consent.

Given our limited powers on the introduction of new treatments, we cannot simply set conditions of the use of add-ons. In January 2019, the HFEA published a consensus statement signed by an additional 10 signatory bodies which sets out the principles of responsible innovation which we believe should guide professionals in the UK. The HFEA is continuing to work on a range of initiatives designed to set standards and inform patients about the use of treatment add-ons in the UK. The HFEA has published information for patients on some of the add-ons being offered in the UK based on an annual review by our independent Scientific and Clinical Advances Advisory Committee of relevant published research. The HFEA has tightened its guidance for clinics to require that any information provided to patients by clinics on the safety and effectiveness of any add-on explicitly references the HFEA website. In future we envisage that new treatments or technologies under development will only be offered to patients outside of a research setting once safety and effectiveness have been demonstrated. More high-quality research including Randomised Controlled Trials, meta-analyses, and follow-up of patients is needed. We want to move towards a more consistent and transparent approach to the use of treatment add-ons in fertility services.

INVITED SESSION

SESSION 56: FRONTIERS IN ANDROLOGY

08 July 2020 Parallel 2 08:30 - 09:30

O-221 Capturing the sperm flagellum: Mechanistic andrology

D.Smith[1]

[1]*University of Birmingham, School of Mathematics, Birmingham, United Kingdom*

O-222 When will we start transplanting prepubertal testis tissue?

C. Wyns[1]

[1]*n/a, n/a, BRUSSEL, Belgium*

Abstract text

Testicular tissue transplantation in humans was already performed back in 1889 by Brown-Sequard to treat hypogonadism. However, grafting the tissue to restore the reproductive potential has never been reported.

Efforts to preserve the fertility of prepubertal boys facing fertility-threatening therapies by cryopreserving their immature testicular tissue have motivated researchers to develop methods to give these young patients a hope to father their own genetic child. In this regard, auto-transplantation of cryostored tissue can be considered if there is no risk of cancer cell contamination of the tissue i.e. not in case of haematological cancers or metastasizing tumours.

The proof of principle of the technique was recently obtained with the birth of GRADY in macaques but many research questions remain before pilot trials can be considered in humans where most often only small amounts of tissue are available for future use.

Xenotransplantation experiments with human prepubertal testicular tissue in nude mice showed an important loss of spermatogonia in grafts, regardless of prior cryopreservation, justifying optimization of the avascular transplantation procedure.

High DNA fragmentation by TUNEL was observed in the first days following grafting suggesting that cell damage is likely due to hypoxia before vascular supply of oxygen to the grafted tissue is in place. In this regard, finding the most appropriate graft size to reduce tissue necrosis and cell loss is a first matter of concern. Only one study compared graft outcome for different tissue sizes and no differences were observed up to 16mm³. Attempts to shorten the ischemic period

before revascularization of the graft were also made by culturing the tissue fragment before grafting in media containing vascular endothelial growth factor (VEGF) as this method increased the number of seminiferous tubules with elongating spermatids in bovine. Unfortunately, this could so far not be confirmed for human prepubertal tissue although a potential benefit was suggested in a report of two cases.

However, controlled local drug delivery of VEGF covering the time period needed for the stabilization of the neo-vasculature could be more efficient. Therefore, nanoparticles (NPs) containing growth factors e.g. VEGF, platelet-derived growth factor (PDGF) and necrosis inhibitors have been developed as well as tissue embedding matrices allowing proper migration of endothelial cells. Higher recovery rates of undifferentiated spermatogonia in autografts were reached in an alginate hydrogel and further improvement was achieved with NPs controlled drug delivery.

Incomplete and abnormal differentiation of spermatogonia in xenografts of human prepubertal testicular tissue was also observed, most likely due the phylogenetic distance between mice and human, but not only. Indeed, graft development seems also influenced by its hormonal environment as autografting experiments in marmosets showed less advanced germ cell development in hemi-castrated than castrated animals. While it was reported that human prepubertal Leydig cells show maturation features based on the presence of key enzymes of steroidogenesis and ultrastructural modifications after xenografting, only few studies have investigated the impact of the hormonal environment on the spermatogonial stem cell niche in grafts. Some questions still need to be addressed: what would be the real impact of the post-chemotherapeutic hypergonadotropic hypogonadic state of candidate patients for autotransplantation on transplant outcome, and would hormone requirements of the transplanted tissue depend on the age of the patient at cryopreservation? Furthermore, and so far, the absence of a valid preclinical model for human prepubertal testicular tissue transplantation precludes to answer the question of the time needed to achieve complete spermatogenesis in grafts and thus the optimal moment for sperm retrieval.

INVITED SESSION

SESSION 57: INTERVENTIONS **EVIDENCE-BASED** **SURGICAL**

08 July 2020 Parallel 3 08:30 - 09:30

O-223 Isthmocele management in infertile patients

O. Donnez[1]

[1]*Elsan, Gynecologic and oncologic surgeon, polyclinique Urbain V, MD, PhD, France*

O-224 Indications for tubal surgery

A. Watrelot[1]

[1]*Hospital Natecia, gynecologic surgery, Lyon, France*

Abstract text

Interest in tubal surgery has decreased progressively in the last decades due to IVF/ICSI results which have dramatically increased in the same time.However IVF access is not easy in many countries and more and more patients wish , if possible, to conceive naturally. Therefore once may ask what is the place for tubal surgery in 2020.

First of all it is important to consider the tubal pathology: the most common pathology is the one affecting the distal part of the tube . Everybody knows today the detrimental effect of hydrosalpinx on fertility and IVF results.

In this case, salpingectomy is often proposed but with a careful selection, a conservative treatment by salpingoplasty may produce very good results in term of spontaneous pregnancy.

A "new" chapter in distal tubal pathology represented by the subtle tubal abnormalities is raising (such as paratubal cysts, sacculation,accessory tubes),and we have more and more evidence that these minor abnormalities should be diagnosed and treated allowing spontaneous pregnancy in more than 50% of cases.

lastly , proximal surgery keeps good indication after sterilization when the patients wishes to restaure her fertility. datas suggest that microsurgical recanalization of the tube through robot or microsurgery gives better outcome in term of pregnancy than IVF/ICSI.

Therefore we consider that tubal surgery, in the hands of trained team, has a role to play ,providing that a good diagnosis is established, a careful selection of patients is made and a complete information is delivered to patients . Nevertheless, nowadays, few surgeons are trained in tubal surgery, except in Far East countries(such as India and China) where tubal surgery is still very popular.

We should probably think to re-promote the teaching of tubal surgery in the initial training of young surgeons, in order not to compete with IVF /ICSI but to provide an additional tool which may be beneficial in some infertile patients

SELECTED ORAL COMMUNICATIONS

SESSION 58: IMPROVING SPERM CRYSOPRESERVATION OUTCOMES

08 July 2020 Parallel 4 08:30 - 09:30

O-225 Does cryopreservation affect live birth outcome in surgically retrieved sperm: – an analysis of a decade of nationwide data

J. Lewin[1], **E. Yasmin**[1]

[1]*University College London Hospital, Gynaecology, London, United Kingdom*

Study question: Is there a difference in live birth rate after ICSI between cryopreserved and fresh surgically-retrieved sperm?

Summary answer: There was no difference in live birth rate between cryopreserved and fresh sperm used in ICSI over 10 years analysed from all UK fertility centres

What is known already: Multiple small studies have been conducted comparing success rates from fresh and cryopreserved surgically-retrieved sperm for use in ICSI. A 2014, meta-analysis showed no significant difference in live birth rate. Most included studies were small and subsequent studies have demonstrated conflicting results. Concerns are raised about the effect of cryo-protectants and freezing/thawing techniques on function and structure of sperm such as DNA fragmentation and acrosomal reaction.

Study design, size, duration: Annual data pertaining to ICSI cycles from all IVF centres in the UK were obtained from the UK Human Fertilisation and Embryology Authority (HFEA). The time period studied included all years from 2007 to 2017 inclusive. Data from 235,296 ICSI cycles were therefore obtained.

Participants/materials, setting, methods: By law all clinics in the UK offering assisted reproductive technology treatments must collect accurate data from all cycles including pregnancy outcome, which is then submitted to the HFEA. We obtained anonymised data from the HFEA on live births per cycle of treatment according to sperm and egg source from 2007-2017. We compared live birth outcomes between cryopreserved and non-cryopreserved sperm from different sources using Chi square, ANOVA and Pearson correlation coefficient where appropriate.

Main results and the role of chance: A total of 235,296 ICSI cycles were performed in the UK between 2007 and 2017 inclusive. Data obtained from these cycles included the number of cycles and live births/ per cycle of treatment for each year categorised by source of egg and sperm (donor or partner) and further divided by method of sperm retrieval (ejaculate, epididymal or testicular) and whether the used sperm were fresh or cryopreserved. Any sub-group with <5 cycles/live births were listed as <5 to protect patient anonymity, so these were excluded from analysis, including all data from 2007 and from donor gamete cycles. 197,836 ICSI cycles were therefore included, which resulted in 61,344 live births over the 10-year period. Of these cycles 184,246 used ejaculated sperm, 5249 epididymal and 8341 testicular, which had live birth rates of 30.93%, 32.94% and 31.60% respectively. There was no difference in the live birth rate between fresh and frozen ejaculated (30.94% vs 29.78%, p=0.208), epididymal (32.59% vs 33.20%, p=0.64) or testicular (31.59% vs 31.64%, p = 0.97) sperm. There was no difference between any of the groups when analysed by one-way

ANOVA (p=0.089), and no statistically significant correlation between the year and the live birth rate for any group.
Limitations, reasons for caution: ICSI cycles using donor oocytes were excluded from analysis due to low numbers. The primary diagnosis (obstructive or non-obstructive azoospermia) was not known in patients undergoing surgical retrieval of testicular sperm and a sub-group analysis between live outcome in obstructive and non-obstructive azoospermia would have provided important clinically relevant information.
Wider implications of the findings: The lack of difference in live birth outcomes in different sources of partner sperm allays some of the concerns about using frozen sperm for ICSI especially in surgically retrieved sperm. This analysis can serve as evidence to counsel patients undergoing surgical retrieval of sperm.
Trial registration number: not applicable

O-226 Novel Technologies for Single-Sperm Vitrification with Cryotop and Cell Sleeper: A Follow-Up Study

Y. Endo[1], S. Mitsuhata[1], M. Hayashi[1], Y. Fujii[1], H. Motoyama[1]
[1]Kurashiki Medical Clinic, IVF Center, Kurashiki, Japan

Study question: Do the novel sperm vitrification devices (Cryotop and Cell Sleeper) efficiently vitrify small numbers of spermatozoa?
Summary answer: Single-sperm recovery was successful with both devices; fertilization and embryo cleavage occurred normally, embryo transfer was successful, and four babies were liveborn.
What is known already: We developed the effective single-sperm vitrification methods by using the nonbiological carriers Cryotop (Kitazato, Tokyo, Japan) and Cell Sleeper (Nipro, Osaka, Japan) and were the first to report the successful delivery of infants from the individually vitrified sperm from a man with nonobstructive azoospermia (Endo et al., 2011, 2012). Since the publication of our reports, however, there have been few reports of live birth after the use of sperm from men with extremely severe male infertility. Because the population of such men is quite limited, insemination with donor sperm is a common option in such cases.
Study design, size, duration: This was a retrospective study. From January 2010 to December 2018, vitrification of small numbers of spermatozoa was performed for 9 men with azoospermia after testicular biopsy and for 3 men with cryoptozoospermic semen. The ICSI treatments were performed in 16 cycles for sperm from Cryotops and 14 cycles for those from Cell Sleepers.
Participants/materials, setting, methods: Vitirification: All motile specimens were vitrified before the day of oocyte pickup. Two to 14 spermatozoa were loaded into cryoprotectant solution in each device (Cryotop and Cell Sleeper), which was placed in liquid nitrogen vapor. Subsequently, they were stored in a cryogenic tank. Warming: On the day of oocyte retrieval, Cryotops and Cell Sleepers were taken out of the tank. Sperm were retrieved with intracytoplasmic sperm injection (ICSI) needles and used in ICSI procedures.
Main results and the role of chance: A total of 475 sperm in 55 Cryotops and 171 sperm in 18 Cell Sleepers were vitrified. During ICSI, 30 Cryotops and 16 Cell Sleepers were warmed, and the sperm recovery rate was 83% (218/264) and 91% (135/148), respectively ($P < 0.05$). After warming, 34% (75/218) of sperm from Cryotops and 15% (20/135) from Cell Sleepers were motile, and the difference was significant ($P < 0.01$). A total of 87 Cryotop sperm and 74 Cell Sleeper sperm were used in ICSI and injected into oocytes individually. The normal fertilization rate was significantly higher in the Cryotop group than in the Cell Sleeper group (48% [42/87] vs. 32% [24/74]; $P < 0.05$), but the rate of embryo cleavage did not differ (88% [37/42] vs. 96% [23/24]). Fresh and frozen embryos from both groups underwent 16 and 10 transfer cycles, respectively, and 16 embryos from the Cryotops group and 11 from the Cell Sleeper group were transferred to patients. The rates of pregnancy were 13% (2/16) with Cryotop group and 20% (2/10) with Cell Sleeper group. Finally, 2 healthy babies from Cryotop group and 2 from Cell Sleeper group were born.
Limitations, reasons for caution: Fewer than 0.7% (12/1837) of patients undergoing oocyte pickup required the novel technologies of single-sperm vitrification during the experimental period in Kurashiki Medical Clinic (Okayama, Japan). Further clinical follow-up studies will provide more information about which device has more clinical benefit for single-sperm vitrification.
Wider implications of the findings: Our findings suggest that Cryotop and Cell Sleeper methods would be suitable and clinically useful for men with severe male infertility who hope to have biological children. Furthermore, our effective sperm storage methods contributed to reduce the time-consuming and exhausting search for individual sperm in the laboratory.
Trial registration number: not applicable

O-227 comparison of permeable cryoprotectant free vitrification by droplet method versus rapid freeze using glycerol in abnormal semen sample

S. Ranganathan[1], S. Reddy[1], M. Daniel[1], S. Srinivasan[1]
[1]Sri Ramachandra Institute of Higher education and Research, Reproductive Medicine, Chennai, India

Study question: Is permeable cryoprotectant free vitrification using droplet method of abnormal semen sample a good alternative to conventional rapid freeze using glycerol?
Summary answer: The permeable cryoprotectant free sperm vitrification protocol tested in this study renders considerably better recovery rates of abnormal semen sample compared to rapid freezing.
What is known already: Semen cryopreservation in contemporary practice is conventional rapid freezing; however, more recently, vitrification has also been used. The rapid freeze method has been associated with a decline in sperm quality with negative effects on both structural and functional sperm features. One of the recently emerged technology within the field of cryobiology is spermatozoa vitrification.This method is based on rapid cooling of cells by immersion into liquid nitrogen and, thereby, is the key to reducing the chance of forming big ice crystals. Vitrification is still yet to be explored, since only limited studies shows its efficacy in abnormal semen sample.
Study design, size, duration: This study included 80 oligozoospermic sperm samples from patients seeking ART treatment between 2018-2019. The effects of permeable cryoprotectant free vitrification protocol on functional sperm quality parameters in comparison to fresh and rapid freeze using glycerol samples were assessed.
Participants/materials, setting, methods: All samples were divided into three aliquots : fresh(F), vitrification, warming(V), rapid freeze(R). Sperm total motility, progressive motility, vitality and spontaneous acrosome reaction were assessed and compared between both the groups.
Main results and the role of chance: Results showed better preservation of sperm features after vitrification compared to conventional rapid freeze. Permeable cryoprotectant free vitrification using droplet method presented a significantly higher percentage of total motile, progressively motile and live spermatozoa compared to rapid freezing. The percentage of sperms undergoing premature capacitation was lower in frozen thawed vitrified samples compared to rapid freeze samples. The total motility in V group was 37.79±11.98 and R group was 36.28±11.90. Progressive motility in V group was 22.01±8.45 and R group was 18.31±8.18. The vitality in V group was 51.56±12.31 and R was 49.95±12.22. Acrosome reacted sperms were significantly high in R group with 43.29±9.96 compared to V group 31.93±8.72. Acrosome intact sperms were significantly high in V group with 65.21±8.86 compared to R group 54.09±9.44. The analysis of sperm quality parameters studies revealed that the V group preserved the sperm quality much better than the R group, indicating that samples are better preserved through vitrification than rapid freezing.
Limitations, reasons for caution: This study has been done only on oligozoospermic sperm samples. It is necessary to compare these results in surgically retrieved sperm samples to evaluate the influence of the application of this methodology in routine clinical practice.
Wider implications of the findings: The sperm vitrification protocol described here guarantees better maintenance of sperm quality parameters than conventional rapid freeze and can prove to be a better and economical alternative to preserve sperm samples from patients seeking ART treatment. Further it opens new doors in IVF and ICSI.
Trial registration number: not applicable

O-228 Sperm banking before gonadotoxic treatment: is it worth the effort?

E. Reiser[1], K. Vomstein[1], T. Kriesche[1], B. Böttcher[1], G. Pinggera[2], B. Toth[1]

[1]Medical University Innsbruck, Department of Gynecological Endocrinology and Reproductive Medicine-, Innsbruck, Austria ;
[2]Medical University Innsbruck, Department of Urology, Innsbruck, Austria

Study question: Do cancer patients already show impaired sperm quality prior to gonadotoxic treatment?
Summary answer: Sperm quality was already impaired prior to gonadotoxic treatment.
What is known already: Cryopreservation of semen is a well-established method as chemotherapy, surgery and radiotherapy have deleterious effects on spermatogenesis. However, patients suffering from malign, as well as benign diseases show impaired semen characteristics already prior to gonadotoxic treatment. Therefore, this study aims to compare semen quality between different cancer types and benign diseases. Furthermore, the usage rate of cryopreserved semen is reported to be as low as 8% for assisted reproductive treatments (ART) and was also analyzed in our study cohort.
Study design, size, duration: Within this retrospective study, the semen quality and the utilization of cryopreserved semen, was assessed in a total of 264 patients suffering from cancer and benign diseases prior to any gonadotoxic therapies. Patients were referred to the Department of gynecological endocrinology and reproductive medicine, Innsbruck, Austria between 01/2008 and 07/2018. Pre-treatment semen analyses were studied and compared in accordance with the WHO guidelines. In addition, the usage of cryopreserved semen for ART was evaluated.
Participants/materials, setting, methods: Pre-treatment semen analyses were studied and compared in accordance with the WHO guidelines in 264 patients with testicular cancer, hematological malignancies and benign diseases prior to any gonadotoxic therapies. In addition, the usage of cryopreserved semen for ART was evaluated.
Main results and the role of chance: Patients with testicular cancer (TM) showed a lower sperm concentration (12 *106/ml) compared to hematological malignancies (HM) (28.5 *106/ml, p=0.0029) and benign diseases (Ben) (29 *106/ml, p=0.0008). Within the TM group, the group of seminomas (8.5 *106/ml) presented the lowest sperm counts. Although most of the individual mean values were within the WHO reference limits, only 38 % of TM and 45 % of sarcoma patients had a normozoospermia. In the other groups (HM, Ben) only 60 % of the patients showed a normozoospermia. No correlation between sperm quality and Ann- Arbor stadium, TSH- values or BMI was present. Only 5 (1.9 %) patients used their frozen semen for a total of 9 ART cycles, which resulted in 5 live births.
Limitations, reasons for caution: The low use rate might be explained by the short follow up time after cryopreservation and patients' age. The storage of semen for fertility preservation is currently not covered by health insurance in Austria compared to other countries, where storage costs play a minor role.
Wider implications of the findings: Depending on the underlying disease, patients show different sperm quality, with TM and HM patients having the lowest sperm concentrations. Due to the low usage rate of cryopreserved semen, a control sperm test should be part of the follow-up cancer care - to avoid costly storage.
Trial registration number: x

SELECTED ORAL COMMUNICATIONS

SESSION 59: NEW MORPHOKINETIC INSIGHTS OF EMBRYO DEVELOPMENT

08 July 2020 Parallel 1 10:00 - 11:45

O-229 Improving embryo selection by development of laboratory-adapted Time-lapse model

I. Blais[1], S. Lahav-Baratz[1], M. Koifman[1], I. Feferkorn[1], M. Dirnfeld[1,2]

[1]Lady Davis Carmel Medical Center, Division of Reproductive Endocrinology and IVF- Department of Obstetrics and Gynecology, Haifa, Israel ;
[2]Technion – Israel Institute of Technology, Ruth and Bruch Rappaport Faculty of Medicine, Haifa, Israel

Study question: Is it applicable to develop morphokineric algorithm for a specific IVF laboratory, can it provide more information for embryo selection in addition to general models?
Summary answer: A model based on laboratory-specific morphokinetics was found to be complementary to the general models and an important tool for improving single embryo selection.
What is known already: Generally applicable algorithms for selection and mainly deselection of embryos from a cohort are routinely used in IVF labs. It was shown that clinical and laboratory conditions influences embryo morphokinetics. In order to avoid the differences between labs, the general algorithms were developed by using large range of cells division timing. It is recommended that laboratories develop a model that uses the specific embryo division timing obtained under local conditions. However, it was not reported whether a specific model can be developed in a lab that embryos were already selected for transfer by using the general models.
Study design, size, duration: During 2013-2018, 12,944 embryos were incubated in our EmbryoScope (Vitrolife), using the general models for embryo selection. There were 1879 KID (known implantation data) embryos, of which, 425 were positive KIDs. For the outcome, we set three endpoints for KID definition: gestational sacs (GS), clinical pregnancy (CP), and live birth (LB). Comparison between positive and negative KID embryos for cell division timings was analyzed separately for ICSI and IVF, at patient's age 18-41 years old.
Participants/materials, setting, methods: Implanted and non-implanted embryos were analyze for statistical differences in cell division timing and cell cycles intervals. We used EmbryoScope Stats software for model building. The timing parameters were tested for their contribution to the scoring in the model. The algorithms were tested for the area under the receiver operating characteristic curve (AUC) in KID embryos for developing Day 2,3 and 5 models. The stability of the algorithms structure was verified by performing calibration-validation procedures.
Main results and the role of chance: Since significant differences in cell division timings were found between implanted and non-implanted embryos, we were able to develop a laboratory-adapted model. The algorithms were developed for selection of Day 2, 3 and 5 ICSI embryos. AUC at most cases were higher than 0.65 which indicates that these models are valid in our laboratory. In addition, these values for AUC were obtained across all GS, CP and LB KID embryos databases tested. An increase in the predictability of the models was observed from Day 2-3 to Day 5 models. AUC test results ranged around 0.658, 0.671 for Day 2, Day 3 respectively and 0.872 for Day 5 model. Our results show that although embryos were selected for transfer using the general models, specific laboratory model is contributory for better selection of embryos. It may improve embryo selection and increase the number of single embryo transfers.

Developing a laboratory-specific model requires many stages of sorting and characterization. It demands accurate annotation and statistical analysis for every parameter alone and in combination, to evaluate which parameter should be included in the model. Many conclusions can be drawn about the model-building process, which may facilitate and improve the process in other laboratories.
Limitations, reasons for caution: Laboratory specific in house model is an important tool that helps selecting embryos for transfer, but should be used with caution and in combination with other selection method such as general models and embryo classical morphology evaluation by the embryologists.
Wider implications of the findings: We recommend developing in-house model for embryo selection in addition to the general models. This may enable single embryo transfers with more confidence, reducing multiple pregnancies and the number of transfers required to achieve pregnancy. The experience we gained in the process may be of assistance to other laboratories.
Trial registration number: Institutional study registration number: 0135-18-CMC

O-230 Trophectoderm specification: compaction, polarisation and inner and outer cells occur simultaneously in the human preimplantation embryo

A. Demtschenko[1], W. Essahib[1], G. Verheyen[2], K. Sermon[3], H. Tournaye[2], H. Van de Velde[1]

[1]VUB, Reim, Brussels, Belgium ;
[2]Uz Brussel, crg, Brussels, Belgium ;
[3]VUB, Rege, Brussels, Belgium

Study question: How is the trophectoderm lineage specified in the human preimplantation embryo?

Summary answer: Outer cells at the fully compacted embryo stage display characteristics of trophectoderm cells such as apical polar distribution of p-ERM and nuclear GATA3/YAP1/TEAD4 protein expression.

What is known already: In mice, the first indication of inner cell mass (ICM) and trophectoderm (TE) segregation occurs three days post fertilisation (dpf3). After compaction, asymmetric divisions give rise to inner and outer blastomeres that ubiquitously express transcription factor TEAD4 and sequester polarity markers (p-ERM), and TE lineage specifiers (YAP1, GATA3) to outer blastomeres. This is considered the onset of TE lineage segregation in the mammalian embryo. Studies regarding this event in the human embryo are absent and extrapolations from the mouse model cannot be a surrogate for studies in the human embryo.

Study design, size, duration: Human embryos used in this study, were donated to research as a surplus of IVF-ICSI treatment at our IVF-center after the cryo-storage expiration date of 5 years with informed consent. The project was approved by the institutional ethical committee and the federal committee for research on human embryos.

Participants/materials, setting, methods: Cryopreserved human 8-cell stage embryos, donated to research, or zygotes created for research after informed consent were warmed (Vit Kit -Thaw, Irvine Scientific, USA) and cultured until dpf5 according to standard laboratory procedures of the associated IVF-clinic. Time course immunofluorescence was performed on fixed embryos at predefined stages for TEAD4, YAP1, GATA3, p-ERM as well as F-actin and Hoechst for imaging (LSM800, ZEISS) and the manual estimation of individual blastomere counts per embryo (ImageJ).

Main results and the role of chance: The compaction process of the human embryo is interrupted by cleavage divisions this results in a fully compacted embryo at dpf4.These fully compacted embryos are comprised of a total of 16±3 cells, n=21, of which 76%, n=16/21 contain 1±1 inner blastomere in their core excluded from the exterior. The nuclei of the outer blastomeres are positive for TE-specifier YAP1 (69±22%, n=8) and begin to co-express TE-transcription factor GATA3 (15±22%, n=8). The apical membranes of the outer blastomeres appear polarised by p-ERM staining (100%, n=8) and TEAD4 is expressed ubiquitously (100%, n=8). While inner cells also express TEAD4 they remain apolar and rarely express YAP1. This inner/outer pattern is maintained in the blastocyst ICM and TE. All nuclei of TE cells of dpf5 blastocysts are positive for YAP1/GATA3 and TEAD4 and apical membranes of TE cells show polar distribution of p-ERM. Whereas the ICM remains apolar, TEAD4 positive, GATA3 negative, and very rarely contains YAP1 positive cells.

Limitations, reasons for caution: In spite of available functional data on the interaction between YAP1 and TEAD4 and their effect on GATA3 expression and embryo development in the mouse and the bovine model, functional experiments in the human embryo still need to confirm the link between polarity, nuclear TEAD4/YAP1/GATA3, TE specification and blastocyst formation.

Wider implications of the findings: The same molecular determinants are involved in the TE lineage segregation of mice and humans, but their expression differs according to the morphological development of the respective species. In contrast to the stepwise model of the mouse, compaction, polarisation and inner and outer cells occur gradually in the human embryo.

Trial registration number: not applicable

O-231 Embryos excluding multinucleated cells during blastocyst formation increase their reprodutive potential

A. MUNUERA PUIGVERT[1], S. Novo[1], L. Almenara[1], A. García-Faura[1], B. Marquès[1], F. García[1], C. Castelló[1], M. López-Teijón[1]

[1]Institut Marquès, Reproductive Medicine Service, Barcelona, Spain

Study question: Has the inclusion or exclusion of blastomeres in multinucleated embryos any effect on embryo development and clinical outcomes on IVF treatments?

Summary answer: Embryos excluding multinucleated cells during blastulation appear to have the capacity for self-correction that allows them to keep intact their ability to become healthy babies.

What is known already: The effects of multinucleation on embryo development and on clinical outcomes are unclear. Some studies have correlated multinucleation with low embryonic development, low implantation potential and aneuploidies increase. However, there is still a debate about transferring multinucleated embryos, as some other studies have observed that multinucleation is equally present in euploid and aneuploid embryos. Moreover, there are live births reported from multinucleated embryos. One of the theories about these discrepancies is the location of the multinucleated cells once the blastocyst is formed. In this way, these cells may be part of the inner cell mass, the trophectoderm or be excluded.

Study design, size, duration: Retrospective study involving 20,779 embryos from 5,621 cycles performed between 2014 and 2019. Two main groups were considered: Control Group (CG;N=16,897), embryos without multinucleation and, Multinucleation Group (MNC;N=3,879), embryos showing at least one multinucleated blastomere. Embryos transferred belonging to MNC Group (N=307) were subdivided according to the multinucleated cell location after blastulation: MNC-1 (N=142), no cells excluded; MNC-2 (N=73), mononucleated cells excluded; MNC-3 (N=46), multinucleated cells excluded. All groups were homogeneous.

Participants/materials, setting, methods: All the embryos were cultured until blastocyst stage using one-step culture media. We used the time-lapse technology to follow up the location of the multinucleated cells and we had to exclude from the study those transferred embryos that could not be monitored (N=46). Single embryo transfer was performed to all patients and the clinical outcome rates were compared between groups performing the statistical analysis Chi-square test.

Main results and the role of chance: Our results showed that despite presenting a significantly lower rate of blastocyst formation (MNC=20.0%; CG=58.0%; p<0.05), the transfer of multinucleated embryos that were able to reach the blastocyst stage at D5/6 had remarkable reproductive success.

Blastocysts that were able to exclude multinucleated cells (MNC-3) achieved higher clinical outcomes respect those including multinucleated cells (MNC-1 and MNC-2). Pregnancy rate in MNC-3 (63.0%) was significantly higher than in the other groups (MNC-1=50.7%; MNC-2=41.1%; p<0.05). The clinical pregnancy rate in MNC-3 (56.5%) was higher than MNC-1 (41.5%) and MNC-2 (32.9%), being statistically significant with MNC-2 (p<0.05).

Miscarriage rate of MNC-3 was lower than the other groups but the differences were not significant (MNC-1=16.2%; MNC-2=9.6%, MNC-3=6.5%).

It was in the live birth rates when MNC-3 (48.9%) showed the highest significant differences regarding the other two groups (MNC-1=24.8%, MNC-2=22.2%; p<0.05).

Moreover, we found that embryos excluding multinucleated cells (MNC-3) reached equivalent clinical outcome results to CG (with 4210 blastocysts transferred): pregnancy rate: 63.0% vs. 60.8%; clinical pregnancy rate: 56.5% vs. 50.6%; live birth rate: 48.9% vs. 34.4%.

Limitations, reasons for caution: There is a wide difference on sample size between MNC and CG groups. 14.7% of the MNC transferred embryos had to be excluded from the study because they could not be well monitored under time-lapse viewer.

Wider implications of the findings: We consider that MNC should be classified according to their ability to locate multinucleated cells. The exclusion of multinucleated cells could be part of some error detection mechanism. Embryos that are able to discard them while reaching the blastocyst stage have the same reproductive potential as blastocyst without multinucleation.

Trial registration number: Doesn't apply

O-232 Deep learning using embryo preimplantation videos can automatically predict the potential of human embryos to blastulate and implant

Y. Ken-Tor[1], N. Zabari[1], A. Szeskin[1], A. Tamar[1], D. Richter[2], Y. Or[3], Z. Shoham[3], A. Hurwitz[4], I. Har-Vardi[2], M. Gavish[1], A. Ben-Meir[4], A. Buxboim[5]

[1]Hebrew university, Computer science, Tel Aviv, Israel ;
[2]Soroka University Medical center, Fertility and IVF Unit- Department of Obstetrics and Gynecology, Beer Sheeva, Israel ;
[3]Kaplan Medical Center, Fertility and IVF Unit, Rehovot, Israel ;
[4]Hebrew University Hadassah Medical Center, Fertility and IVF Unit- Department of Obstetrics and Gynecology, Jerusalem, Israel ;
[5]Hebrew university, The Alexender Grass Center for Bioengineering- School of computer Science and Engineering, Tel Aviv, Israel

Study question: Does the morphodynamic data in preimplantation human embryo videos can be analyzed using deep learning algorithms to predict the potential to reach blatulation and implantation.

Summary answer: Blastulation prediction on test-set embryos, reached area under the ROC curve 0.73 at 72 hour. prediction on of day-5 transferred test-set embryos, outperformed manual KIDScore™-D5.

What is known already: In IVF treatments, early identification of embryos with high implantation potential is required for shortening time to pregnancy while avoiding clinical complications caused by multiple embryo pregnancy. Current classification tools are based on manually annotated morphological and morphokinetic parameters. However, manual annotation introduces inter-observer and intra-observer variability and provides a discrete representation of preimplantation development while ignoring dynamic features that may be associated with embryo quality. Deep learning AI, which offers a powerful toolbox for carrying out automated and standardized classification tasks involving expansive datasets, is gradually incorporated into the health care system worldwide and IVF clinics specifically.

Study design, size, duration: 16,000 3D videos of preimplantation embryos were annotated for morphological and morphokinetic parameters. 6,200 were blastulation-labelled and > 5,500 were implantation-labelled. Each embryo video file was associated with maternal information and clinical metadata. The classification was evaluated using fivefold cross-validation and using an uncontaminated test set consisting of 20% of the labeled embryos. Generality was verified via leave-one-clinic-out and leave-one-age-group out cross-validation.

Participants/materials, setting, methods: Embryos were cultured in nine time-lapse incubators during the past six years in four medical centers across Israel. We developed fully automated and standardized classifiers by training time point sensitive deep neural networks directly on video frames. Using machine learning we combined the frame scores into a single embryo score for blastulation or implantation prediction. Using SHAP values we identified key time points for prediction and impactful video frames.

Main results and the role of chance: Blastulation prediction on test set embryos increased monotonically with the time of prediction measured from ICSI, reaching area under the ROC curve (AUC) 0.73 at 72 hours, 0.88 at 96 hours and 0.94 at 110 hours. Blastulation prediction was further demonstrated using high-quality embryos that reached 8 cells.

KID predictive strength increased with time of prediction as evaluated for the same cohort of day-5 transferred test-set embryos. AUC increases slowly from 48 to 84 hours and more rapidly from 84 hours onward. Our deep learning prediction is as accurate as KIDScore-D3 on day-3 and more accurate than KIDScore-D5 on day-5 as evaluated for the same test-set embryos.

Both blastulation and implantation results were consistent with fivefold cross-validation, leave one-clinic-out and leave one-age-group out from the training session, supporting generality.

Using SHAP analysis we identified key temporal points that can direct blastulation and implantation prediction without including the rest of the video. we trained again the embryo-learning blastulation and implantation classifiers using only the key temporal points. The new models reached comparable AUC for both goals supporting the importance of a few time points.

Limitations, reasons for caution: Prediction accuracy is limited due to lacking critical information about endometrial receptivity and using a homogenous dataset of embryos that were retrospectively preselected for transfer according to established morphological and/or morphokientic criteria.

Wider implications of the findings: Deep learning provides full automation and standardization of embryo classification and improves accuracy. Our classifiers mark the first step towards the development of a decision support tool. This framework opens the door for clinical implementation of deep learning classification tools that will improve conception rates while shortening time to pregnancy.

Trial registration number: not applicable

O-233 Direct cleavage in late stages of embryo progession does not affect in vitro development, ploidy nor the reproductive potential of human embryos

J. Masso Hernaez[1], S. Novo[1], V. Moens[1], À. García-Faura[1], B. Marquès[1], F. García[1], C. Castelló[1], M. López-Teijón[1]

[1]Instituto Marques, Reproductive Medicine Service, Barcelona, Spain

Study question: What are the effects of direct cleavage (DC) on embryo development and preimplantation genetic testing (PGT) results?

Summary answer: The results for embryos that present DC and develop into good quality blastocysts (GQB) are equivalent to those that do not present DC.

What is known already: After the appearance of time-Lapse technology, various studies based on morphokinetics have identified the presence of direct cleavage in 14% - 26.1% of all evaluated embryos. These embryos present a significant reduction in blastocyst formation, euploidy rate and implantation potential compared to control groups without DC. However, depending on the cycle where DC is observed, the impact may not be so negative.

Study design, size, duration: A retrospective study including 11168 embryos from 3175 cycles between January 2018 to October 2019. Two groups were defined: a DC-negative group (DC-) containing 10051 embryos without DC in any of their division cycles and a DC-postive group (DC+) including 1117 embryos with at least one DC in any of their first three cycles. DC+ group was subdivided into DC+1 (n=493), DC+2 (n=476) and DC+3 (n=148) based on the cycle in which DC was observed.

Participants/materials, setting, methods: For all cycles intracytoplasmatic sperm injection technique was performed and the resulting embryos were cultured in time-lapse incubators to blastocyst stage using one-step culture media. Blastocyst, good quality blastocyst (GQB), multinucleation and ploidy rates were compared between both groups, with and without DC. Furthermore, groups DC+1, DC+2 and DC+3 were also compared among each other. The chi-square test was used for statistics.

Main results and the role of chance: At least one DC event was observed in 10% of embryos on culture day 2 or 3. This rate was lower compared to values published previously by others laboratories. The *in vitro* development rate of DC+ embryos up to day 5/6 (42.4%) was significantly lower than the DC- embryos (73,6%) ($p<0.05$). Presence of multinucleated cells was more frequent in DC+ embryos (487/1117; 43.6%) than DC- embryos (1411/10051; 14%) ($p<0.05$). GQB rate was significantly lower the earlier DC happened (DC+1 46/493; 9.3%, DC+2 106/476; 22.3%, DC+3 66/148; 44.6%; $p<0.05$). It is noteworthy that DC+3 GQB rate was similar to the control group (5298/10051; 52.7%) ($p=0.0601$). When only considering embryos biopsied on day 3, DC+ embryos had lower euploidy rates (8/84; 9.5%) than DC- ones (316/1036; 30.5%) ($p<0.05$). However, on day 5/6 biopsy, the euploidy rate was equivalent (DC+ 30/82; 36.6% vs DC- 497/1300; 37.9%; $p=0.8569$), as was pregnancy rate (DC+ 7/12; 58.3% vs DC- 228/325; 70.2%; $p=0.5786$) and ongoing pregnancy rate (DC+ 5/12; 41.7% vs DC- 185/325; 56.9% $p=0.4531$). Differences in mosaicism rates were not significant (DC+ 10/82; 12.2% vs DC- 237/1300; 18,2%; $p=0.2168$).

Limitations, reasons for caution: Due to their decreased potential depending on the moment of DC, the number of DC+ embryos biopsied and/or transferred was lower compared to DC-. A larger sample in the study group is necessary to confirm the above results.

Wider implications of the findings: Embryos with early DC+ should never be the first option for transfer. From our results, in cases where PGT is indicated, biopsy must be performed on day 5/6. If an embryo is capable of developing into a GQB, events that are linked to bad prognosis may have been resolved.

Trial registration number: not applicable

O-234 Application of the embryo-uterus statistical model for prediction of implantation after day 3 embryo transfer by using time-lapse morphokinetics and female age

E. Van Marion[1], E. Baart[1], E. Van Santbrink[2], M. Avo Santos[2], J. Laven[1], M. Eijkemans[3]

[1]Erasmus University Medical Centre Rotterdam, Division of Reproductive Endocrinology and Infertility- Department of Obstetrics and Gynaecology, Rotterdam, The Netherlands ;

[2]Reinier de Graaf Groep, Division of Infertility- Department of Obstetrics and Gynaecology, Voorburg, The Netherlands ;

[3]University Medical Centre Utrecht, Biostatistics and Research Support, Utrecht, The Netherlands

Study question: Can we develop a robust and clinically applicable time-lapse morphokinetic model, which predicts embryo implantation potential after single embryo transfer (SET)?

Summary answer: A prediction model based on the time interval between the four- and five-cell stage and female age showed adequate performance on an independent data set.

What is known already: Over the past years, time-lapse embryo culture is increasingly used as a semi-quantitative tool to research the timing of embryo development and the correlation with implantation. Studies with different sample sizes and statistical approaches have led to either centre specific or generally applicable models to support embryo selection. The predictive capability of time-lapse selection algorithms may be influenced by patient characteristics, type of data included in the analysis and the used statistical methods. Earlier studies excluded double embryo transfer (DET) cycles of which only one embryo implanted, introducing bias in the data.
Study design, size, duration: This is a retrospective study of couples (n= 707) undergoing an *in vitro* fertilization (IVF) cycle with or without intracytoplasmic sperm injection (ICSI) at the Erasmus University Medical Centre between January 2012 and June 2019. Embryo transfer was either SET or DET. This resulted in 785 transferred embryos that reached at least the five-cell stage. Embryo selection was not guided by time-lapse morphokinetics but was performed on day 3 according to classic morphological criteria.
Participants/materials, setting, methods: Embryos were cultured in the EmbryoScope™ and retrospectively annotated for developmental time points up to the 8-cell stage. The final prediction model was established by using the Embryo-Uterus model (EU) (Roberts, Stat Med 2007:p156) with the number of gestational sacs as the outcome variable. Correlations between embryos that implanted simultaneously were included in the model. The model was externally validated on time-lapse data from 1269 fresh embryo transfers performed at the Reinier de Graaf hospital.
Main results and the role of chance: We performed manual backward selection using $P < 0.3$ for inclusion in a multivariable EU-regression analysis with the following parameters: female age, time to three-cell stage (t3), time-interval between the two- and the three-cell stage (t3-t2), time to four-cell stage (t4), time-interval between the four- and the five-cell stage (t5-t4), and the ratio of the time-interval between the three- and the five-cell stage and the two- and the five-cell stage (t5-t3/t5-t2). As the relationship between these parameters and gestational sacs was non-linear, they were included using cubic splines. All variables were entered in the Embryo part of the model. This resulted in a final model including female age and t5-t4, yielding the probability of clinical pregnancy after SET, with the optimal timing of t5-t4 between 10-14 hours. An embryo that needs less time between t5-t4 shows a steeper decrease in implantation potential than if it needs longer. This was consistent for all female ages. The area under the receiver operating characteristic curve (AUC) of the final model was 0.624. The model was capable of predicting clinical pregnancy in the external dataset with an AUC of 0.639 and showed adequate calibration with a slope of 1.235.
Limitations, reasons for caution: Although the model shows good calibration and validation, prospective validation is needed to establish clinical applicability. Embryo transfer (ET) was performed on day 3. For clinics performing day 5 ETs the model can be relevant for poor responders or patients that do not want to culture until day 5.
Wider implications of the findings: We were able to minimize selection bias by analysing both SETs and DETs irrespective of the number of implanted embryos and selecting embryos for transfer by morphology alone. This model can be used to counsel couples on their pregnancy chances before embryo transfer, and as a decision tool for cryopreservation.
Trial registration number: not applicable

O-235 Does calcium ionophore treatment have any effect on embryo kinetics?

I. Keles[1], B. Balaban[2], A. Isıklar[2], S. Ertaş[2], B. Urman[2], B. Ata[1], K. Yakin[3]

[1]Koc University Hospital, Assisted Reproduction Unit, ISTANBUL, Turkey ; [2]American Hospital of Istanbul, Assisted Reproduction Unit, Istanbul, Turkey ; [3]Koc University, Obstetrics and Gynecology, Istanbul, Turkey

Study question: Is oocyte activation with calcium ionophore associated with morphokinetic parameters in cleavage and blastocyst stage embryos?
Summary answer: Embryos that were grown from activated oocytes did not show any difference in terms of morphokinetic parameters.
What is known already: Alterations in calcium signalling may be one of the underlying reasons for defects in cell growth and clevage. It has been shown in humans that calcium fluctuations were detected with a peak shortly before cell division and these calcium oscillations disappeared in arrested embryos. Clinical use of calcium ionophore treatment after intracytoplasmic sperm injection (ICSI) demonstrated an improvement in fertilization, cleavage and blastulation rates in couples with fertilization failures or severe male factor infertility. However, there is no data available on the morphokinetic evaluation of embryos developed following activation.
Study design, size, duration: This retrospective cohort study included 798 embryos from 484 couples undergoing ICSI from 2018-2020. We used propensity score matching to compare the morphokinetic parameters of embryos that were developed following ICSI with or without oocyte activation via ready to use calcium ionophore.
Participants/materials, setting, methods: The indication for oocyte activation was either severe male factor infertility or previously failed/low fertilization(≤ 25%). After excluding women ≥40-year of age, the study group comprised 121 women and the control group 363 . Groups were re-analysed according to female age and ICSI indication . All embryos were cultured in a timelapse incubator in the same single step medium and annotated at t2, t3, t4, t5, t8 and tB time points by a single embryologist.
Main results and the role of chance: The mean female age was 33.7 years (19-40). Morphokinetic measurements showed normal distributions. The mean annotation time points for "t2, t3, t4, t5, t8 and tB" in the control versus study group were in the following order: 26.8 vs 26.9, 35,0 vs 35.2, 38.4 vs 38.3, 46.6 vs 47.0, 56.1 vs 55.5, 104.9 vs 103.8 hrs ($p>0.05$ for all). When adjusted according to female age and ICSI indication, no difference was detected between two groups for any time points.
Limitations, reasons for caution: This was a single center retrospective study reporting on a limited number of ICSI cycles in which calcium ionophore treatment was deemed necessary.
Wider implications of the findings: Calcium ionophore treatment of oocytes to improve fertilization and/or clinical outcome in selected patients does not have a significant impact on embryo kinetics.
Trial registration number: Not Applicable

SELECTED ORAL COMMUNICATIONS

SESSION 60: LONG TERM HEALTH, OBSTETRICS AND NEONATAL OUTCOMES RELATING TO INFERTILITY TREATMENT

08 July 2020 Parallel 2 10:00 - 11:45

O-236 Pregnancy, Delivery, and Neonatal Outcomes Among Women with Congenital Adrenal Hyperplasia: A population based study on 9.1 million pregnancies

A. Badeghiesh@gmail.com[1], H. Baghlaf[2], E. Suarthana[1], M. Dahan[1]

[1]Mcgill University, Obstetric and Gynecology department, Montreal, Canada ; [2]University of Toronto, Obstetrics & Gynecology, Toronto, Canada

Study question: What are the pregnancy, delivery and neonatal outcomes in congenital adrenal hyperplasia (CAH) patients?
Summary answer: CAH patients have a two-fold higher risk of; chorioamnionitis, maternal infection and cesarean section also fetal increases in small for gestational age and congenital malformation
What is known already: Congenital adrenal hyperplasia (CAH) is a cluster of inherited enzymatic defects of adrenal steroid biosynthesis. Deficiencies of each enzyme required in the steroid biosynthesis pathway are well known, and these deficiencies are all inherited as autosomal recessive disorders. Women with CAH have decreased fertility because of oligo-ovulation. Conception requires a combination of proper therapeutic compliance, careful endocrine monitoring, and often ovulation induction. There are significant gaps about pregnancy, delivery and neonatal outcomes among CAH patients. The purpose of this study is to investigate these outcomes.
Study design, size, duration: We conducted a retrospective population-based cohort study utilizing data from the Health Care Cost and Utilization Project-Nationwide Inpatient Sample database (HCUP-NIS) over 11 years from 2004 to 2014. We created a cohort of all deliveries between 2004 and 2014 inclusively. Within this group, all deliveries to women with CAH were identified as part of the study group (n=299), and the remaining deliveries were categorized as non-CAH births and comprised the reference group (n=9,096,489).

Participants/materials, setting, methods: Analysis was performed to identify the prevalence of pregnant women with CAH over the study duration. Demographic and clinical characteristics were compared between women with and without CAH using Chi-square test. All confounding variables were adjusted for using multivariate logistic regression, based on any significant differences between the two groups generating adjusted odds ratios (aOR).

Main results and the role of chance: There were 9,094,499 deliveries during the study period. 299 pregnant women were found to have CAH. Compared to the control group, CAH subjects were likely to be: older (p<0.001), white (p<0.001), have higher incomes (p<0.001), have private medical insurance (P<0.001), be obese (p<0.001), have a previous cesarean section (p<0.001), chronic hypertensive (7.4% versus 1.8%, p<0.001), pre-gestational diabetics (5% versus 0.9%, p<0.001), and have thyroid disease (11.7% versus 2.5%, p<0.001). Chorioamnionitis was higher in CAH compared to controls (aOR 2.67, 95% CI 1,17 -6.06). The rate of caesarian section (aOR 2.10, 95% CI 1,44 – 3.07) and maternal infection (aOR 2.63, 95% CI 1,22 -5.63) were higher in CAH. Rates of pregnancy induced HTN, preeclampsia, eclampsia, gestational diabetes, preterm delivery, preterm premature rupture of membranes and postpartum hemorrhage did not differ between CAH group and the control group.

At birth, 8% and 2.2% of the neonates were found to be small for gestational age (SGA) in CAH and control groups respectively (aOR 3.37 95% CI 1.86 - 6.11). Congenital anomalies were encountered in 2.7% of the CAH group compared to the control group (0.4%) (aOR 5.24 95% CI 2.31 - 11.90)

Limitations, reasons for caution: This retrospective analysis utilizes an administrative database, with its inherent limitations. Significant medical history or adverse pregnancy outcomes may be more often reported in patients with more significant conditions or outcomes

Wider implications of the findings: CAH patients are at risk of: chorioamnionitis, maternal infection and cesarean section. CAH is associated with small gestational age infants possibly related to chronic maternal steroid use. The rate of congenital malformations likely related to elevated androgens in female offspring and chronic glucocorticoid used in pregnancy was about 2.7%.

Trial registration number: not applicable

O-237 Vitamin D concentration at term in newborn-mother pairs with and without polycystic ovary syndrome: association with perinatal outcome

M. Kollmann[1], B. Obermayer-Pietsch[2], G. Pregartner[3], E. Lerchbaum[4], S. Feigl[1], P. Klaritsch[5]

[1]Division of Obstetrics and Maternal Fetal Medicine, Department of Obstetrics and Gynecology- Medical University of Graz, Graz, Austria ;
[2]Endocrinology Lab Platform- Department of Obstetrics and Gynecology and Division of Endocrinology and Metabolism, Department of Internal Medicine- Medical Uniersity of Graz, Graz, Austria ;
[3]Institute for Medical Informatics- Statistics and Documentation IMI, Medical University of Graz, Graz, Austria ;
[4]Division of Endocrinology and Metabolism, Department of Internal Medicine- Medical University of Graz, Graz, Austria ;
[5]Division of Obstetrics and Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Graz, Austria

Study question: Do mothers with/without PCOS have different vitamin D levels at term, how are vitamin levels reflected in their offspring, and are vitamin levels associated with an adverse perinatal outcome?

Summary answer: Vitamin D levels did not differ significantly in women with/without PCOS and their respective offspring. Vitamin D deficiency was not associated with adverse perinatal outcome.

What is known already: Studies suggest that non pregnant women with PCOS may be at elevated risk of vitamin D deficiency. Furthermore, there is evidence suggesting that vitamin D may also play an important role during pregnancy. Data regarding vitamin D deficiency during pregnancy in PCOS patients and its association with perinatal outcome is scarce.

Study design, size, duration: Prospective cross-sectional study

Participants/materials, setting, methods: We included 79 women with PCOS according to the ESHRE/ASRM 2003 definition and 354 women without PCOS and an ongoing pregnancy ≥ 37 + 0 weeks of gestation who gave birth in our institution between March 2013 and December 2015. Maternal serum and cord blood vitamin D levels were analysed at the day of delivery.

Main results and the role of chance: Maternal vitamin D levels did not differ significantly in women with PCOS and without PCOS (p=0.998), nor did the vitamin D levels of their respective offspring (p=0.692). Vitamin D deficiency (< 20 ng/mL) was found in 26.9% and 22.5% of women with and without PCOS (p=0.430). There was a strong positive correlation between maternal and neonatal vitamin D levels in both investigated groups. Linear regression estimates of cord blood vitamin D levels are about 77% of serum vitamin D concentrations of the mother. Compared to healthy controls, the risk for maternal complications was increased in PCOS women (48% versus 65%; p=0.009), while there was no significant difference in neonatal complications (22% versus 22%; p=1.0). However, vitamin D levels were similar between mothers and infants with and without perinatal complications.

Limitations, reasons for caution: Fetal cord blood was used for the analysis of fetal hormonal levels.

Wider implications of the findings: Although the share of women and infants with vitamin D deficiency was high in women with and without PCOS, it seems that the incidence of adverse perinatal outcome was not affected. The long-term consequences for mothers and infants with a vitamin D deficiency have to be investigated in future studies.

Trial registration number: NCT02106676

O-238 Early ovarian ageing and long-term health consequences: Is number of oocytes harvested in ART associated to an earlier and increased risk of age-related diseases?

M.W. Christensen[1], U.S. Kesmodel[2], K. Kirkegaard[3], H.J. Ingerslev[2]

[1]Århus University Hospital, Obstetrics and Gyneacology, Aarhus N, Denmark ;
[2]Aalborg University Hospital, Fertility Unit, Aalborg, Denmark ;
[3]Aarhus University Hospital, Obstetrics and Gyneacology, Aarhus N, Denmark

Study question: Do young women with early ovarian ageing defined as unexplained, repeatedly few oocytes harvested in ART have an increased risk of age-related diseases?

Summary answer: At follow-up young women with idiopathic early ovarian ageing had an increased risk of age related diseases compared to young women with normal ovarian ageing.

What is known already: Early and premature menopause is associated with an increased risk of cardiovascular diseases (CVD), osteoporosis and death. Identifying women at risk may thus allow early preventive health initiatives. Repeatedly few oocytes harvested in well stimulated assisted reproductive technology (ART) cycles is a likely predictor of advanced menopausal age when seen in young women and may thus serve as an early marker of accelerated general ageing. Oocyte harvest in ART as a measure of ovarian ageing and thus as a risk predictor of age-related morbidity and mortality has not been investigated previously.

Study design, size, duration: A register-based national historical cohort study. Young women (≤ 37 years) having their first ART-treatment in a Danish fertility clinic (public or private) during the period 1995-2014 was divided into two groups dependent on ovarian reserve status: early ovarian ageing(EOA) (n=1,234) and normal ovarian ageing(NOA) (n=18,614). Number of oocytes harvested in first and subsequent cycles was used as a marker of ovarian reserve. Several national registers were applied to assess morbidity and mortality.

Participants/materials, setting, methods: EOA was defined as ≤ 5 oocytes in minimum two well-stimulated cycles and NOA as ≥8 oocytes in minimum 1 cycle. Known causes influencing the ovarian reserve (endometriosis, surgery, chemotherapy etc.) was reason for exclusion. Primary outcome was overall-disease risk defined as either: CVD, osteoporosis, type-2 diabetes, cancer, all-cause death, Charlson Comorbidity index, cataract, Alzheimer's or Parkinson's disease or early retirement benefit. Cox regression models were used to assess the disease risk after first ART-cycle.

Main results and the role of chance: Median follow-up time from first ART-cycle to first disease event was 6.1 years (10/90 percentile 1.0/12.9) and 6.7 years(10/90 percentile 1.0/14.1) in the EOA -and NOA group respectively. Women with EOA had an increased risk of overall- disease when compared to women with a normal oocyte yield (Adjusted HR 1.26, 95 % CI 1.10;1.43). Stratifying on diseases categories, the EOA group had a significantly increased risk for cardiovascular diseases (adjusted HR 1.39, 95 % CI 1.15; 1.67),

osteoporosis (adjusted HR 2.36, 95 % CI 1.48;3.74), Charlson comorbidity index (Adjusted HR 1.28, 95 % CI 1.06;1.54) and early retirement benefit (adjusted HR 1.52, 95 % CI 1.06;2.19).

Limitations, reasons for caution: Due to register limitations we were unable to identify the reason why no oocytes had been collected in case of cancelled cycles and we may have missed women with the most severe forms of EOA. Neither did we have information on the total doses of gonadotropin given in each cycle.

Wider implications of the findings: These findings indicate that oocyte yield may serve as marker of later accelerated ageing when unexpected, repeatedly few oocytes are harvested in young women. Counselling on life-style factors as a phrophylactic effort against cardiovascular and other age related diseases may be essential for this group of women.

Trial registration number: The study was approved by the Danish Data Protection Agency (J.nr 1-16-02-319-14)

O-239 The worldwide epidemic of Vitamin D deficiency: are we contributing by using inaccurate and unreliable measurement methods?

E.E. Lara Molina[1], J.M. Franasiak[2], A. Devesa-Peiro[3], M. López-Nogueroles[4], M. Florensa[5], M. Martin[5], D. Amoros[6], A. Ballesteros[7], A. Pellicer[8], P. Diaz-Gimeno[9]

[1]IVI RMA Global Barcelona, Egg Donation, Barcelona, Spain ;
[2]IVI RMA Global New Jersey, Chief Medical Officer of IVIRMA America, New Jersey, U.S.A. ;
[3]Valencia University, Gynecology and Obstetrics, Valencia, Spain ;
[4]Biomedical Research Institute La Fe, Analytical Unit, Valencia, Spain ;
[5]IVI RMA Global Barcelona, IVF laboratory, Barcelona, Spain ;
[6]IVI RMA Global Barcelona, General laboratory, Barcelona, Spain ;
[7]IVI RMA Global Barcelona, Reproductive Unit, Barcelona, Spain ;
[8]IVI RMA Global Rome, IVI RMA Global President, Rome, Italy ;
[9]Biomedical Research Institute La Fe, IVI Foundation IVI RMA Global, Valencia, Spain

Study question: Is Vitamin D deficiency diagnosis biased by which measurement technique is utilized?

Summary answer: 25-hydroxyvitamin D (25OHD) serum concentrations are significantly lower when measured via Enzyme-Linked Immunosorbent Assay (ELISA) compared to Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

What is known already: Vitamin D deficiency is widely reported in general population and in women undergoing ART, but lack of accuracy when measuring its metabolites remains as an unresolved issue. 25OHD is the most abundant vitamin D metabolite in the circulation, and is proposed to be the best indicator of vitamin D status. Although LC-MS/MS for serum 25OHD measurement is theoretically a more accurate and reliable technique than immunoassay-based methods, these are faster and less laborious, and are more commonly used for Vitamin D assessment in the general practice.

Study design, size, duration: 34 healthy women participating in our egg donation program were included during four months in this prospective, non-interventional cohort study. Serum samples were collected for quantification of 25OHD concentrations with the use of LC-MS/MS procedure and with ELISA. 25OHD levels according to IOM guidelines (<20, 20-30, and >30 ng/mL) were evaluated according each method results.

Participants/materials, setting, methods: Serum was obtained in each subject, and then separated into two samples. 25OHD concentrations in one of the samples were measured via LC-MS/MS using a UPLC-TQ-S Xevo Waters system with a Waters Acquity BEH C18 (1,7µm 2,1 x100mm) column. A Vitamin D Enzyme-Linked Immunosorbent Assay (ELISA) kit (ab213966) Abcam for the quantitative determination of 25OHD was used for the other sample. A paired Wilcoxon test was performed for contrasting the mean differences between both techniques.

Main results and the role of chance: All the cases were included and studied during autumn and winter months. None of them had taken vitamin D oral supplements during the last six months before sampling. Mean value for 25OHD concentrations in serum was 36.96±15.78 ng/ml when measuring via LC-MS/MS, and significantly lower when ELISA method was used (20.74 ± 21.73 ng/ml, p-value=1.255e-05). According to IOM guidelines, there was no Vitamin D deficiency in our studied population of 34 healthy women on reproductive age when LC-MS/MS is utilized, and most of them were in the sufficiency range of >30 ng/ml (n=23; 67.65%). In contrast, this population could be classified in the insufficiency range according to mean values obtained via ELISA method, most of them showing concentrations bellow <20ng/ml (n=23; 67.65%).

Limitations, reasons for caution: The study population was a rather homogenous group of young, healthy women. These findings need to be confirmed in a larger, more diverse patient population**.**

Wider implications of the findings: Utilizing ELISA to measure serum vitamin D levels results in an overestimation of vitamin D deficiency and may explain the increased prevalence in the population. Thus, LC-MS/MS should be considered as a more reliable procedure to measure Vitamin D in research and clinical practice.

Trial registration number: not applicable

O-240 Polycystic Ovary Syndrome as an independent risk factor for gestational diabetes and hypertensive disorders of pregnancy: A population-based study on 9.1 million pregnancies

G. Mills[1], A. Badeghiesh[2], E. Suarthana[2], H. Baghlaf[2], M. Dahan[3]

[1]McGill University, Ob GYN, Montreal, Canada ;
[2]McGill University, Ob Gyn, Montreal- QC, Canada ;
[3]McGill University, McGill University Health Center Reproductive Center, Montreal- QC, Canada

Study question: Does polycystic ovary syndrome (PCOS) confer an independent risk for the development of gestational diabetes (GDM), gestational Hypertension (GHTN), and pre-eclampsia (PEC)?

Summary answer: PCOS confers a two-fold higher risk of developing GDM, a 50% increased risk for of developing GHTN, and a 30% increased risk of developing PEC.

What is known already: Despite significant evidence of an increased prevalence of maternal pregnancy complications in women with PCOS, there remain significant gaps in understanding how PCOS affects the development of GDM, GHTN, and PEC. This is most likely due to the complex, multifactorial etiology of PCOS, its range of potential confounders for pregnancy complications, and the variable methodology of studies that have been conducted. To date, the largest meta-analysis on this subject includes 11, 565 women with PCOS analyzed for their risk of GDM and 5896 patients analyzed for their risk of PEC.

Study design, size, duration: We conducted a retrospective population-based cohort study utilizing data from the Health Care Cost and Utilization Project-Nationwide Inpatient Sample database (HCUP-NIS) over 11 years from 2004 to 2014. We created a cohort of all deliveries between 2004 and 2014 inclusively. Within this group, all deliveries to women with PCOS were identified as part of the study group (n=14,882), and the remaining deliveries were categorized as non-PCOS births and comprised the reference group (n=9,081906).

Participants/materials, setting, methods: The analysis was performed to identify the prevalence of pregnant women with PCOS over the study duration. Baseline clinical and demographic characteristics between both groups were compared. Logistical regression analyses were conducted to explore associations between PCOS and maternal metabolic outcomes through the estimation of odds ratio (OR) and 95% confidence intervals (CI). The regression models were adjusted for the potential confounding effects of maternal demographic, preexisting clinical characteristics, and concurrently occurring characteristics.

Main results and the role of chance: At baseline, more pregnant women with PCOS had obesity (22.3% vs. 3.5% p<0.001), chronic hypertension (8.4% vs. 1.8% p<0.001), pre-gestational diabetes (4.1% vs. 0.9% p<0.001), and thyroid disease (12.6% vs. 2.4% p<0.001). Women with PCOS were more likely to have undergone IVF treatment (2.4% vs. 0.1% p<0.001), have multi-gestation pregnancies (5.9% vs. 1.5% p<0.001), and more multiple gestations in the PCOS cohort were the result of IVF treatment than the Non-PCOS cohort (12.3% vs. 2.3% p<0.001).

In singleton pregnancies, women with PCOS were more likely to develop gestational diabetes (aOR 2.17, 95% CI 1.99-2.35), pregnancy-associated HTN (aOR 1.41 95% CI 1.29-1.54 p<0.001), gestational hypertension (aOR 1.48, 95% CI 1.31-1.66), pre-eclampsia (aOR 1.31, 95% CI 1.15-1.50), and superimposed pre-eclampsia (aOR 1.34, 95% CI 1.07-1.67) after controlling for confounding effects (age, race, income level, insurance type, obesity, IVF use, previous C/S, chronic HTN, pre-gestational diabetes, thyroid disease, smoking,

and recreational drug use). In multiple gestation pregnancies, PCOS only conferred an increased risk of developing GDM (aOR 2.33 95% CI 1.92-2.83 p<0.001). However, there was a non-significant trend towards an increased risk for developing pregnancy-associated HTN (aOR 1.92 95% CI 0.99-1.42 p=0.058) in the multiple gestations group.

Limitations, reasons for caution: This retrospective analysis utilizes an administrative database, relies on the accuracy of individuals reporting and coding data. Remote diagnoses of PCOS or mild phenotypes may not have been reported. Significant medical history and/or adverse pregnancy outcomes may be more often reported in patients with more significant conditions and outcomes.

Wider implications of the findings: PCOS is an independent risk factor for the development of GDM, GHTN, and PEC. It is important to consider the risk of all other co-existing metabolic conditions in women with PCOS, as these risks are additive and can significantly increase the risk of adverse complications in pregnancy.

Trial registration number: not required

O-241 GNRH antagonist administration to decrease risk of OHSS in GNRH agonist cycles triggered with HCG

G. Mills[1], S.Y. Dahan[1], M.H. Dahan[1]

[1]McGill University Health Centre, Obstetrics and Gynecology, Montreal, Canada

Study question: Does administration of a gonadotropin-releasing hormone antagonist after HCG trigger in GnRH agonist cycles with significant stimulation reduce the risk of developing ovarian hyperstimulation syndrome?

Summary answer: Addition of GnRH antagonist for 7-10 days to cabergoline treatment after hCG-trigger in agonist cycles reduces the risk of moderate/severe OHSS compared to cabergoline alone

What is known already: In ART cycles that cannot or have not undergone a GnRH agonist trigger, such as in GnRH agonist cycles or when OHSS signs and symptoms appear in low risk cycles after administration of hCG, secondary risk reduction strategies can be employed. Currently, the two main secondary OHSS risk reduction strategies are to avoid fresh embryo transfer to prevent endogenous production of hCG, and administration of the dopamine agonist, cabergoline. However, when using these strategies, the risk of moderate and severe OHSS remains present.

Study design, size, duration: A retrospective cohort study of 171 IVF patients at the McGill University Health Centre treated with GnRh-agonist protocols and with unexpected exuberant response to stimulation from 2011-2019 was performed. Women received triggering with 5000IU urinary hCG or 250mcg recombinant hCG, and were converted to freeze all cycles.

Participants/materials, setting, methods: Patients were allocated to one of two groups. The NO-ANT group (n=123) received cabergoline (Pfizer, Montreal, Canada) 0.5 mg daily, and the ANT group (n=48) received cabergoline treatment and GnRH antagonist (ganirelix, Orgalutran® 0.25 mg/0.5 ml, Merck, Canada). Treatment in both groups lasted for 7 days starting on the oocyte retrieval day. If moderate or severe OHSS occurred, GnRH antagonist was continued for 10 days. No fresh embryo transfers were performed.

Main results and the role of chance: 171 patients were identified as being at risk for developing OHSS after receiving an hCG trigger as part of an agonist cycle. There were no differences between the two groups (No-ANT vs. ANT, respectively) in terms of; age (36.2±2.8 vs. 35.8±3.2 years *p=0.42*), basal serum FSH levels (8.1±2.3 vs. 7.9±2.3 IU/L *p=0.61*), or AFC (12.2±2.4 vs. 11.6±1.9, *p=0.12*). The ANT group had more oocytes collected (18.6 vs. 17.1 *p=0.03)*, more 2PN embryos (14.3 vs. 12.2 *p<0.001)*, and more frozen blastocysts (4.4 vs. 3.9 *p=0.04)*.

The NO-ANT group had more cases of moderate and severe OHSS (52% vs 25% *p = 0.001*, and 25% vs 10% *p = 0.03,* respectively), more occurrences of free pelvic fluid (74% vs. 35% *p<0.001),* and more bloating and discomfort (91% vs. 65% *p<0.001)* than patients in the ANT group. The number of peritoneal ascites drainages/cases of severe OHSS were lower in ANT group (0.4 vs. 1.2 *p=0.01*).

The ANT group had lower serum hemoglobin (14.2±1.4 g/dl vs. 15.1±1.3 g/dl *p<0.001)*, higher serum albumin (29.4±3.4g/L vs. 23.6±2.9g/L *p<0.001)*, higher serum sodium (132.9±2.6 vs. 132.0±2.2 mEq/L *p=0.02*), and lower serum potassium levels (4.6±0.7 vs. 5.2±1.0 mEq *p<0.001*) than the NO-ANT group.

Limitations, reasons for caution: As a retrospective cohort study, there may be some amount of selection and misinformation bias that cannot be accounted for. This study has a relatively small sample size, suggesting the possibility of a type I error. However, given the levels of significance between group outcomes, however, this risk remains low.

Wider implications of the findings: Addition of GnRH antagonist for 7-10 days to routine cabergoline treatment after an hCG trigger in agonist cycles may reduce the risk of developing moderate and severe OHSS compared to cabergoline alone. Further studies are warranted to determine if GnRH-antagonists can be used as a treatment for OHSS once started.

Trial registration number: Not Applicable

O-242 Artificially prepared frozen embryo transfer cycles are associated with an increased risk of preeclampsia.

C. Roelens[1], A. Racca[1], S. Mackens[1], L. Van Landuyt[1], L. Gucciardo[2], M. De Vos[1], H. Tournaye[1], C. Blockeel[1]

[1]UZ Brussel, Centre for Reproductive Medicine, Jette, Belgium ;
[2]UZ Brussel, Department of Obstetrics and Prenatal Medicine, Jette, Belgium

Study question: Is the development of preeclampsia (PE) following frozen embryo transfer (FET) related to the method of endometrial preparation?

Summary answer: Hormonal replacement therapy (HRT) FET cycles are associated with a significantly higher risk of developing PE when compared with natural cycle (NC) FET.

What is known already: PE is a multisystem disorder encountered in approximately 2-5% of pregnancies and a leading cause of maternal and perinatal mortality and morbidity. Although assisted reproductive technology (ART) is an established risk factor for the development of PE, the exact underlying mechanism has not been elucidated. Recent studies have shown a correlation between endometrial preparation before FET and the development of PE during a subsequent pregnancy. The impact of the absence of a corpus luteum on the hormonal environment and vascular adaptations in early pregnancy has been hypothesized as a possible contributing factor in the development of PE after HRT-FET cycles.

Study design, size, duration: We performed a retrospective cohort study at a tertiary university-based hospital encompassing 537 unique patients who had a pregnancy following FET between 2010 and 2019 and delivered in the same institution. 324 patients underwent FET in a NC while 213 performed a HRT cycle. A sample size calculation was performed and showed that 396 patients were needed to detect with 90% power a difference of 8.9% between study and control group.

Participants/materials, setting, methods: The primary outcome was the incidence of PE defined as the development of hypertension after 20 weeks of gestation associated with one or more new-onset conditions: proteinuria, maternal or uteroplacental dysfunctions. Multivariable regression analysis was performed to account for confounding factors knowing to affect the occurrence of PE, including: NC versus HRT, body mass index (BMI), ethnicity, previous history of hypertension during pregnancy and mean arterial pressure (MAP) at the first prenatal consultation.

Main results and the role of chance: No difference was found in the following baseline demographic and clinical characteristics between NC and HRT FET cycles: maternal age, BMI, nulliparity, smoking habits, associated medical conditions, partners age, developmental stage of the embryo, multiple pregnancy, term at delivery and birth weight. Patients suffering from PCOS and ovulation disorders were more commonly represented in the HRT group as compared with the NC group (41.3% versus 6.2%, p<0.001). African ethnicity was more frequent in the HRT group (15.9% versus 8.9% for HRT and NC respectively, p=0.021). Patients undergoing a HRT cycle delivered more often by caesarean section as compared with the NC group (42.7% versus 29%, p=0.001). MAP at the first prenatal consultation was significantly higher in HRT FET cycles (87.9 mmHg ± 17.8 and 82.9 mmHg ± 14.9 for HRT and NC respectively, p=0.001).

The incidence of PE was significantly lower in NC FET cycles (3.7% versus 11.3% for respectively NC and HRT FET cycles, p=0.001). Univariate and multivariate logistic regression analyses were performed in order to account for relevant confounding factors. After confounder adjustment the incidence of PE was significantly lower in the NC FET group (NC versus HRT: aOR 0.35, 95% CI 0.17-0.74, p=0.006).

Limitations, reasons for caution: Although the study adjusted for potential confounding factors, the results remain limited by the retrospective nature of the study and its potentially associated bias.
Wider implications of the findings: The higher incidence of PE in HRT versus NC FET cycles found in our population adds further weight to the existing data on this topic. These significant findings should urge practitioners to preferentially perform FET in a natural cycle instead of a HRT cycle in ovulatory patients.
Trial registration number: not applicable

O-243 High anti-Müllerian hormone levels are associated with preterm delivery in patients with polycystic ovary syndrome

K. Hu[1], F.T. Liu[2], R. Li[2]

[1]Peking University Third Hospital, Center for Reproductive Medicine- Department of Obstetrics and Gynecology, Beijing, China ;
[2]Peking University Third Hospital, Center for Reproductive Medicine, Beijing, China

Study question: Is the serum AMH levels associated with the risk of preterm delivery in PCOS patients?
Summary answer: High anti-Müllerian hormone levels are associated with preterm delivery in patients with polycystic ovary syndrome.
What is known already: Anti-Müllerian hormone (AMH) levels are higher in patients with polycystic ovary syndrome. Accumulating evidence indicates that AMH has an impact on the physiology of the female reproductive system. Increased AMH levels in PCOS patients are associated with PCOS severity and greater ovarian stimulation during IVF treatment. During pregnancy, serum AMH levels are also higher in PCOS than non-PCOS patients, and the elevated AMH levels observed in PCOS patients have an impact on the endocrine system of the fetus.
Study design, size, duration: This is a retrospective cohort study. A total of 25165 in vitro fertilization (IVF) cycles with AMH data performed from January 2017 to July 2018 in Peking University Third Hospital were available for analysis in this study..
Participants/materials, setting, methods: Among the 25165 cycles, 10718 were fresh embryo transfer (ET) cycles, and 14447 were frozen-thawed embryo transfer (FET) cycles. There were 136 preterm deliveries and 1777 term deliveries in the fresh ET group and 145 preterm deliveries and 1685 term deliveries in the FET group. In PCOS patients, 423 term deliveries and 45 preterm deliveries.
Main results and the role of chance: Serum AMH levels were not different between the term delivery and preterm delivery groups in the entire cohort (3.8 vs 4.1 ng/mL, P >0.05). In patients diagnosed with PCOS, those with preterm delivery had higher AMH levels than were found in those with term delivery (9.3 vs 6.9 ng/mL, P <0.01). Preterm deliveries predominated in PCOS patients with AMH levels above the 75th percentile (9.75 ng/ml) (adjusted P <0.0001, adjusted OR=4.0 (95%CI 1.94, 8.08), adjusted for age, BMI, indicated delivery associated complications, embryo transfer number, cesarean or vaginal delivery, fresh ET or FET, gravidity times, parity times, and sex of the infant) and frozen-thawed embryo transfer (FET) patients with AMH levels higher than the 90th percentile (10.10 ng/ml) (adjusted P <0.05, adjusted OR=2.0 (95%CI 1.16, 3.36), adjusted for age, BMI, indicated delivery associated complications, diagnosis (PCOS or non-PCOS), embryo transfer number, cesarean or vaginal delivery, fresh ET or FET, gravidity times, parity times, and sex of the infant). Indicated-delivery associated complications and male infants were risk factors for preterm delivery (adjusted P =0.01, adjusted OR=3.0 (95%CI 1.29, 6.87), adjusted P =0.02, adjusted OR=2.3 (95%CI 1.18, 4.58)). Serum AMH levels were not significantly associated with gestational diabetes or pre-eclampsia.
Limitations, reasons for caution: The main limitation of this study is its retrospective study design and lack of sample size calculation. Some patients with repeated IVF failure may get pregnant more than one year after AMH measurement, which may be different from the exact AMH levels during the cycle of successful pregnancy.
Wider implications of the findings: Our results will guide clinicians to better manage the process of pregnancy in these patients. It will be interesting for further studies to investigate the potential mechanisms underlying these effects.
Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 61: UNDERSTANDING SPERMATOGENESIS BEYOND HISTOLOGY

08 July 2020 Parallel 3 09:50 - 11:55

O-244 Spermiation in mouse is controlled by RARA-mediated gene repression

M. Teletin[1,2], N. Vernet[3], B. Feret[3], M. Klopfenstein[3], W. Bourguet[4], P. Germain[4], P. Weber[5], M. Mark[1,2], N.B. Ghyselinck[3]

[1]1Institut de Génétique et Biologie Moléculaire et Cellulaire IGBMC- CNRS UMR7104 - INSERM U1258- 1 rue Laurent Fries- BP 10142- F-67404 Illkirch CEDEX, Functional genomics and cancer, Strasbourg, France ;
[2]Hôpitaux Universitaires de Strasbourg HUS LBDR CECOS- 1 place de l'Hopital- Strasbourg-, Cecos-Laboratoire de biologie de la reproduction, Strasbourg, France ;
[3]Institut de Génétique et Biologie Moléculaire et Cellulaire IGBMC- CNRS UMR7104 - INSERM U1258- 1 rue Laurent Fries- BP 10142- F-67404 Illkirch CEDEX, Functional genomics and cancer dept, Illkirch, France ;
[4]Centre de Biochimie Structurale CBS- CNRS UMR 5048 - INSERM U1054- 29 rue de Navacelles- F-34090, Biochimie Structurale, Montpellier, France ;
[5]Laboratoire de Biométrie et Biologie Evolutive LBBE- CNRS UMR5558- UCB Lyon 1- Bâtiment Grégor Mendel- 43 Boulevard du 11 novembre 1918- F-69622- Villeurbanne, Biologie Structurale, Lyon, France

Study question: What is the role of nuclear receptor (NRs) and their interaction with cofactors in mouse spermiation?
Summary answer: Our data establish that gene repression mediated in Sertoli cells by NCoR-bound RARA/RXRB heterodimers is instrumental to spermiation in mouse.
What is known already: The activity of nuclear receptors is thought to be mediated by interaction with coactivators or corepressors. In the absence of their cognate ligands, it is admitted that NRs bound to the DNA response elements located in their target genes recruit corepressors and inhibit mRNA expression. The physiological impact of NR-mediated gene repression is poorly characterized. We have shown previously that loss-of-function mutants of RARA or RXRB display spermiation defects.
Study design, size, duration: To address this question, we have generated and analyzed the phenotypes of mice expressing a mutant form of *all-trans* retinoic acid (ATRA) receptor alpha (RARA) which specifically disrupts corepressor binding without affecting agonist-dependent coactivator binding (RARA I396E mutation). We have additionally generated mice in which *NCor1* and *NCor2* were knocked out specifically in Sertoli cells.
Participants/materials, setting, methods: Histological analysis of testes from RARA-I396E expressing mice and also of testes from NCor1 and NCor2 compound double mutants in Sertoli cells were performed together with in situ hybridization techniques, immunofluorescence and transcriptome analyses.
Main results and the role of chance: We demonstrate here that this specific mutations impair spermiation (i.e., the release of mature spermatids by the seminiferous epithelium (SE) of the testis), a hallmark of the phenotype observed upon ablation of RARA or of RXRB in Sertoli cells. Moreover, the combined, Sertoli cell-specific, ablation of the two genes encoding the corepressors NCor1 and NCor2 mimics the spermiation defect observed in RARA-I396E expressing mice. Transcriptome analysis revealed deregulation of a specific set of genes, some of which may control spermatid release. This strongly suggests that gene repression mediated in Sertoli cells by NCoR-bound RARA/RXRB heterodimers is controlling spermiation. It is likely that this repressing activity occurs when the level of ATRA available in the SE is low, which corresponds to stages I to VI of the SE cycle, just prior to spermiation.
Limitations, reasons for caution: Since our present results were obtained in mice, their relevance for human spermatogenesis remains to be demonstrated.
Wider implications of the findings: Pharmacological studies strongly support a role of the ATRA signaling pathway in human spermatogenesis. The evidence provided in this work might suggest that changing RARA activity by

pharmacological approaches in humans might be used to help patients with spermiation defects and to design new male contraceptive strategies.
Trial registration number: Not Applicable

O-245 MicroRNA-targeting in spermatogenesis: Over-expressions of microRNA-23a/b-3p and its affected targeting of the genes ODF2 and UBQLN3 in sperm of patients with oligoasthenozoospermia

M. Abu-Halima[1], B. Ayesh[2], L. Becker[1], M. Hammadeh[3], A. Keller[4], M. Eckart[5]

[1]Saarland University, Institute of Human Genetics, Homburg/Saar, Germany ; [2]al-Aqsa University, Department of Laboratory Medical Sciences, Gaza, Palestine ; [3]Saarland University, Department of Obstetrics and Gynecology- IVF and Andrology Laboratory, Homburg/Saar, Germany ; [4]Saarland Univsersity, Chair for Clinical Bioinformatics, Saarbrücken, Germany ; [5]University of Saarland, Institute of Human Genetics, Homburg/Saar, Germany

Study question: To investigate whether microRNA-23a/b-3p targets the genes *ODF2* and *UBQLN3* and whether this targeting impacts expression levels of *ODF2* and *UBQLN3* in patients with oligoasthenozoospermia.
Summary answer: The over-expression of microRNA-23a/b-3p and lower-expression of *ODF2* and *UBQLN3* genes are associated with male subfertility.
What is known already: Spermatogenesis is the process of male germ cell proliferation and differentiation within the testes. In this complex and highly regulated process, many genes are involved, the expression levels of which are strongly or partially coordinated by microRNAs (miRNAs). MiRNAs are small, non-coding RNAs that are involved in the post-transcriptional regulation of gene expression. Transcriptome analysis shows that hundreds of genes are expressed exclusively or predominantly in male germ cells including *ODF2* and *UBQLN3*, which play a crucial role during spermatogenesis and/or sperm function. However, the expression regulation of these two genes is still unclear.
Study design, size, duration: A total of 86 men were included in the study, including 43 oligoasthenozoospermic men who attended the IVF center for infertility treatment at Saarland University, Germany and 43 age-matched normozoospermic volunteers served as controls. Reverse transcription-quantitative PCR (RT-qPCR), Northern blot, and dual luciferase assay were used to validate the over-expression of microRNA-23a/b-3p and lower-expression of ODF2 and UBQLN3 genes. The study was conducted between 2019 – 2020, at the Institute of Human Genetics.
Participants/materials, setting, methods: Total RNA, including miRNA was isolated from sperm of oligoasthenozoospermic (n=43) and normozoospermic men (n=43). RT-qPCR was used to detect the expression levels of microRNA-23a/b-3p and *ODF2* and *UBQLN3* genes. In silico prediction and dual-luciferase assays were performed to confirm the potential links between the over-expression of microRNA-23a/b-3p and lower-expression of *ODF2* and *UBQLN3* genes. Correlation analyses of the miRNA and mRNA expression levels were done for clinical sperm parameters.
Main results and the role of chance: The expression levels of microRNA-23a/b-3p were significantly up-regulated and *ODF2* and *UBQLN3* genes were significantly down-regulated in oligoasthenozoospermic men compared with age-matched normozoospermic men as determined by RT-qPCR. Using dual-luciferase assays, *ODF2* and *UBQLN3* genes were identified as direct targets of microRNA-23a/b-3p. Mutations in the microRNA-23a/b-3p binding site within the 3′UTRs (3′untranslated regions) of *ODF2* and *UBQLN3* genes resulted in abrogated responsiveness to microRNA-23a/b-3p. Correlation analysis highlighted that sperm count, motility, and morphology was negatively correlated with microRNA-23a/b-3p and positively correlated with the lower expression level of *UBQLN3*, while *ODF* lower expression level was positively correlated with sperm motility.
Limitations, reasons for caution: Despite the negative correlation between the over-expression of microRNA-23a/b-3p and the lower-expression of *ODF2* and *UBQLN3* genes, further validation of these results is needed by an increased number of studied men and by a confirmation of the protein expression by Western blotting.
Wider implications of the findings: Findings suggest that the over-expression of microRNA-23a/b-3p or the lower-expression of *ODF2* and *UBQLN3* genes are associated with oligoasthenozoospermia and male infertility, probably through influencing sperm count, motility, and morphology. This study lay the groundwork for future studies focused on investigating therapies for male infertility.
Trial registration number: Ha195/11

O-246 Relevance of spermiogenic maturation of the male gamete through the epididymal journey

A. Melnick[1], A. Parrella[1], M.S. Wang[1], D. Tavares[1], M. Haddad[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Which area of the epididymis yields spermatozoa with the highest capacity for supporting embryonic development?
Summary answer: The cauda epididymis yields spermatozoa with optimal spermiogenic maturity, resulting in superior fertilization, embryo implantation, and delivery rates.
What is known already: Epididymal sampling is the preferred surgical treatment for men with obstructive azoospermia. The preferred site of retrieval is the caput, which is believed to yield the largest number of spermatozoa with superior kinetic characteristics. Only a few studies have described the fertilization competence of spermatozoa retrieved from the caput, corpus, and cauda regions of the epididymis.
Study design, size, duration: Between 2011 and 2019, 46 men diagnosed with obstructive azoospermia (OA) underwent surgical retrieval of spermatozoa from different areas of the epididymis. Spermatozoa were subsequently used for ICSI cycles with the patients' female partners (≤37 yrs old). Clinical outcome was compared among the different sources of spermatozoa.
Participants/materials, setting, methods: A total of 36 specimens from caput, 7 from corpus, and 3 from cauda were retrieved. Semen parameters were compared among the three sources, and spermatozoa were injected by ICSI in oocytes from the female partners. Fertilization, implantation, and delivery rates were assessed and compared for all the three epididymal locations. Unpaired t and Fisher's Exact tests were used to compare the outcomes. P values <0.05 were considered statistically significant.
Main results and the role of chance: A total of 46 men were divided in three groups according to the source of spermatozoa used: caput, corpus, and cauda. Maternal age was comparable among the three groups (32.0 ± 4, 32.1 ± 4, and 35.0 ± 1, respectively). Average and standard deviation of spermatozoa concentration was 35.1± 39 in the caput, 28.7±29 in the corpus, and 30.2± 59 x106/ml in the cauda.

The mean of motility was 15.8±15% in the caput, rising to 40.1±29.4% in the corpus (P<0.01) and decreasing to 7±11.8% in the cauda (P<0.05). The mean numbers of injected oocytes were 12.7, 11, and 12.5, respectively (P=NS). The fertilization rate was 65.8% with caput, 77.6% with corpus (P<0.05), and 88% (P<0.05) with cauda spermatozoa.

The implantation rate also progressively increased distally through the regions of the epididymis. In the caput, the implantation rate was 34.8%, rising to 44.4% and 57.1% in the corpus and cauda, respectively. The same trend was observed for delivery rates. In cycles using caput spermatozoa, the delivery rate was 48.4% with 5.8% pregnancy loss.

In cycles using corpus spermatozoa, the delivery rate was 75%, with no pregnancy loss. Ultimately, the cycles with cauda had the highest delivery rate, with 100%.
Limitations, reasons for caution: This novel study was performed on a small number of subjects. While we limited female age to ≤37 years, it is not possible to exclude all confounding factors with certainty. In men with OA, it is not always possible to choose the site of surgical sampling
Wider implications of the findings: We have found evidence that the epididymis retains an important role in spermiogenesis. This has been confirmed by the higher fertilization competence of spermatozoa retrieved from the cauda epididymis. Confirmation of these findings in a larger study population may serve as a guide for epididymal sperm retrieval.
Trial registration number: not applicable

O-247 Effect of malignancy on semen quality: An analysis of 3371 patients

E. Yasmin[1], T. Lukaszewski[1], E. Williamson[2]

[1]University College London Hospital, Obstetrics and Gynaecology, London, United Kingdom ;
[2]University College London Hospital, Reproductive Medicine, London, United Kingdom

Study question: How does malignancy and different diagnoses affect semen quality as compared with World Health Organisation (WHO) thresholds of semen analysis in fertile men?
Summary answer: A wide range of malignancies adversely affect semen quality led by testicular cancer and followed by bowel cancer, myeloma and leukaemia.
What is known already: The WHO (2010) published a set of reference values for semen analyses based on the assessment of fertile men with the threshold determined by fifth centiles. The WHO guideline is widely used to determine normality of semen analysis. Observational studies have shown the deleterious effect of malignancy on semen quality. Most studies have small cohorts and larger studies are limited to testicular cancer and haematological malignancies. One of the larger analysis of 4480 cases included both cancer and benign systemic diseases. This study demonstrated poorer sperm quality in different cancers but did not include lung, bowel, myeloma and prostate cancer.
Study design, size, duration: This is a retrospective observational study of single semen analysis prior to oncology treatment in 3371 patients. The data spans a 35 year period (1984-2019). All the samples were collected in a single centre. Only major groups of diagnoses were included in the analysis. Very small groups of heterogenous malignancies were excluded from the study.
Participants/materials, setting, methods: Semen analysis was performed in patients referred for fertility preservation and after they consented to sperm cryo-preservation. All patients were post pubertal. Semen analysis was based on the standardised protocol of the centre. The diagnoses, semen parameters and frozen samples were entered into a secure database. The groups were based on diagnosis. Distribution of the semen parameters at 5th, 10th, 25th, 50th, 75th and 95th was carried out and compared with the WHO references.
Main results and the role of chance: Of the 3371 patients, the majority (66%) were 20-39 yrs with 15.5% under 20yrs. Nearly a third of patients had testicular cancer (33%) and over a quarter had lymphoma (28%). Sarcoma was the third largest group (15%) followed by leukaemia (9%), prostate cancer (6.5%), brain tumour (4%), bowel cancer (3.5%), myeloma (1.5%), lung cancer (0.5%) and melanoma (0.5%). Oligozoospermia (<15mill/ml) was observed in nearly half (46.5%) of patients with testicular cancer followed by bowel cancer (43%), myeloma (41%) and leukaemia (40%). Azoospermia was noted in 4-12% of patients across all diagnoses bar melanoma. The centile distribution of sperm count, sperm concentration, motility and volume revealed normal parameters (as per WHO threshold at 5th centile) only at 50th centile in testicular, brain and bowel cancer, leukaemia and melanoma. Normal values were seen at 25th centile in lymphoma, lung cancer and sarcoma. The threshold of normal was noted at 10th centile in the melanoma group. The study highlights that semen quality is significantly diminished across all diagnoses. The centile distribution of the patients did not match the distribution in fertile men on which the WHO thresholds are based in any diagnosis. Semen quality was significantly affected in bowel, lung and prostate cancer.
Limitations, reasons for caution: A single sample of semen was used for analysis and therefore variation was not accounted for. Period of abstinence could not be standardised as the samples had to be collected at short notice in the context of fertility preservation.
Wider implications of the findings: The findings of the study can be used to inform clinicians and counsel patients presenting for fertility preservation. The study extends information about semen quality across wider diagnoses. The study also provided baseline information for comparison with semen analyses after treatment and in remission.
Trial registration number: Not applicable

O-248 Conditioned media of Sertoli cell cultures induced prepubertal mice spermatogonial cells differentiation in vitro, using methylcellulose system, to express high levels of acrosin and protamine.

M. Huleihel[1], S. Naor[2], E. Lunenfeld[3]

[1]Ben-Gurion University of the Negev, The Shraga Segal Dept. of Microbiology & Immunology, Beer - Sheva, Israel ;
[2]Ben-Gurion University of the Negev, The Shraga Segal Dep. of Microbiology-Immunology and Genetics- The Center of Advanced Research and Education in Reproduction CARER- Faculty of Health Sciences-, Beer-Sheva, Israel ;
[3]Soroka Medical Center and Faculty of Health Sciences- Ben-Gurion University of the Negev, Dep. OB/GYN and The Center of Advanced Research and Education in Reproduction CARER-, Beer-Sheva, Israel

Study question: Do Sertoli cells secrete factors that may induce proliferation and differentiation of mouse spermatogonial cells to meiotic and post-meiotic stages in vitro?
Summary answer: Addition of Sertoli cells conditioned media to spermatogonial cells in vitro, using methylcellulose culture system induced their development to cells that express acrosin and protamine.
What is known already: Sertoli cells (SCs) are active in the process of spermatogenesis and are in a direct contact with spermatogonial cells during their development from spermatogonial stem cells to sperm. Endocrine factors such as follicle stimulating hormone induce SCs to produce factors crucial for spermatogenesis. In addition, SPGCs are involved in the regulation of SC function. SCs produce different factors that affect the proliferation of the spermatogonial cells such as glial-derived nerve growth factor (GDNF), stem cell factor (SCF), leukemia inhibitory factor (LIF) and others. However, the factors that are involved in the differentiation of the spermatogonial cell are not yet clear.
Study design, size, duration: Seven-day-old mice were used to isolate cells from the seminiferous tubules by 2-step enzymatic digestion. Sertoli cell cultures were prepared by culturing the STCs for 3 days in 37 ^{0}C in 5% CO_2 incubator. Thereafter, the cells were treated with hypotonic shock to eliminate the residual germ cells, and re-culture in DMEM media containing 10% KSR. After over-night the media were replaced by fresh which was collected after 8 hours and stored at -80 ^{0}C.
Participants/materials, setting, methods: Isolate cells from the seminiferous tubules (STCs) which contain spermatogonial cells were cultured in vitro in methylcellulose culture system which contained GDNF, LIF, SCF with or without conditioned media (CM) which was collected from SC cultures. The effect of this CM on the development of spermatogenesis in vitro was evaluated by examining the presence/expression of markers of pre-meiotic (VASA, GFR-alpha, SALL4), meiotic (BOULE) and post-meiotic (ACROSIN, PROTAMINE) stages by immunostaining or by qPCR analysis.
Main results and the role of chance: Our results showed that isolated cells from seminiferous tubules of 7-day-old mice contained only pre-meiotic cells (VASA presence in around 8% of cells representing spermatogonial cells), but not meiotic or post-meiotic cells. These spermatogonial cells proliferated in vitro, in methylcellulose culture system, in the presence of GDNF, LIF and SCF to pre-meiotic (VASA presence in around 18% of cells), meiotic (BOULE in around 12% of cells) and post-meiotic cells (ACROSIN in around 8% of cells) after 4-5 weeks of culture (control system). Addition of 10% or 40% (v/v) of SC conditioned media to the spermatogonial cells in the culture did not significantly affect their proliferation of premeiotic cells compared to the control system. However, addition of these SC conditioned media to the spermatogonial cells in vitro significantly increased their development to the presence of acrosin in around 20% of the cells and around 1.5 fold of increase in the RNA expression. Furthermore, an increase in the RNA expression of protamine around 3 fold was found compared to the control system.
Limitations, reasons for caution: The study was performed in mice system and the relevant to human need to be confirmed.
Wider implications of the findings: This is the first demonstration that SCs produce factors that could induce development of spermatogonial cells to post-meiotic cells in vitro. These differentiating factors present in SC conditioned media need to be identified. Our results may assist in using the in vitro system for male fertility preservation in the future.
Trial registration number: Hub Reproduction, Faculty of Health Sciences, Ben-Gurion University

O-249 Appropriate Response of Fallopian Tube Cell Line to Sperms with DNA Fragmentation through Modulating Specific Cytokines

S.O. Mousavi[1], R. Mohammadi[2], F. Amjadi[3], S. Aghajanpour[2], M. Eslami[1], R. Aflatoonian[2]

[1]Faculty of Advanced Science and Technology- Tehran Medical Sciences- Islamic Azad University., Department of Genetics, Tehran, Iran ;
[2]Reproductive Biomedicine Research Center- Royan institute for Reproductive Biomedicine- Tehran- Iran., Department of Endocrinology and Female infertility-, tehran, Iran ;
[3]Iran University of Medical Science- Tehran- Iran., Department of Anatomical Science-, tehran, Iran ;
[4]Faculty of Advanced Science and Technology- Tehran Medical Sciences- Islamic Azad University- Tehran- Iran., Department of Genetics-, tehran, Iran

Study question: How sperms with DNA fragmentation can cause inflammation and influence the host defense?
Summary answer: Some investigations suggest that sperms with DNA fragmentation can change fallopian tube response with alteration in specific cytokines expression.
What is known already: Normal physiological homeostasis in the female reproductive tract needs appropriate regulation of inflammation through cytokines expression. Cytokines are fundamental compartments of immune system. Interaction of sperms with the fallopian tube in the female reproductive tract has an important role in different stages of pregnancy from fertilization to successful delivery. Investigations has shown that specific cytokines such as SPP1, MIF and C5 have relevance in host homeostasis and defense against pathogens.
Study design, size, duration: 30 patients who considered unexplained infertile were selected as donors of semen samples with normal features. After washing sperms, they were categorized to two groups of normal and abnormal DFI by TUNEL assay.

We also cultured fallopian tube epithelial cell into the culture flasks containing DMEM/F12 with 10% FBS medium up to 70% of confluence.
Participants/materials, setting, methods: Fallopian tube epithelial cells were co-incubated with sperms for 24h. after washing cells and extracting the RNA, we synthetized the cDNA. The control group was fallopian tube epithelial cells without sperm. Finally, the mRNA expression levels of the cytokines were evaluated by PCR Array and compared between 3 groups (control, normal DFI, abnormal DFI, n=3×10)**.**
Main results and the role of chance: Our data are agreeing with previous researches which show that cytokines play critical role in sperm interaction with female reproductive tract.

C5 expression in the abnormal DFI group was significantly higher than the control, however there was no significant difference between other groups.

Furthermore, SPP1 expression was higher in normal DFI group than the control, although it was significantly less than the abnormal DFI group. Finally, it is notable that MIF had no significant changes between groups (P<0.05).
Limitations, reasons for caution: Obtaining semen samples due to ethical and logistical issues was the major limitation of this study. Furthermore, *in vitro* co-incubation of fallopian tube epithelial cells with sperms may not directly mimic the *in vivo* interaction.
Wider implications of the findings: According to our data, DNA-fragmented sperms seem to be established as pathogens in female reproductive system and significantly change these cytokines in fallopian tube which prevent fertilization events in advance to other reproductive process.
Trial registration number: not applicable

O-250 A lack of DNA methylation covariation between human blood and sperm make it unlikely to mediate intergenerational inheritance of acquired traits

F. Asenius[1], S.J. Marzi[2], T.J. Gorrie-Stone[3], A. Brew[4], Y. Panchbaya[5], E. Williamson[6], L. Schalkwyk[3], V. Rakyan[4], D.J. Williams[1]

[1]University College London, UCL EGA Institute for Women's Health, London, United Kingdom ;
[2]Imperial College London, Department of Brain Sciences, London, United Kingdom ;
[3]University of Essex, School of Biological Sciences, Colchester, United Kingdom ;
[4]Queen Mary University of London, The Blizard Institute, London, United Kingdom ;
[5]University College London, UCL Genomics, London, United Kingdom ;
[6]University College London Hospital, Fertility & Reproductive Medicine Laboratory, London, United Kingdom

Study question: How does DNA methylation co-vary between matched samples of human whole blood and spermatozoa, and does obesity influence this DNA methylation covariation?
Summary answer: DNA methylation in blood and spermatozoa is clearly distinct, with little evidence of covariation between the tissues. We identified one cross-tissue obesity-associated DNA methylation site.
What is known already: Paternal obesity and T2DM have been associated with an increased risk of fathering low birth weight offspring. Human obesity and T2DM are associated with altered DNA methylation in blood. Animal studies suggest that obesity and insulin resistance are associated with DNA methylation changes in spermatozoa, and that these could mediate intergenerational effects. Such findings are lacking in humans. The largest previous study of DNA methylation in matched human sperm and blood had only 8 participants.
Study design, size, duration: Genomic DNA was extracted from matched samples of semen and blood from 68 lean and 22 obese males. DNA methylation at ~850,000 CpG sites across the genome was analysed using the Illumina MethylationEPIC array to test for 1) interindividual covariation in DNA methylation between blood and sperm, 2) consistent obesity-associated DNA methylation differences across both tissues, 3) obesity altered tissue-covariation, and 4) to compare spermatozoal DNA methylation to that of ~6,000 somatic tissue samples.
Participants/materials, setting, methods: Males of proven fertility were recruited from the University College London Hospital antenatal clinic. All participants provided informed consent and were phenotyped with regards to cardiovascular health.

DNA methylation was measured using the Illumina MethylationEPIC array at UCL Genomics and DNA methylation data was analysed using the wateRmelon package in R.

DNA methylation data from ~6,000 somatic tissue samples was obtained from the Gene Expression omnibus and analysed using the bigmelon package in R.
Main results and the role of chance: There were significant mean DNA methylation differences between blood and sperm at the majority (64%) of the interrogated 704,356 probes ($P < 9 \times 10^{-8}$). At 62% of these, sperm was relatively hypermethylated. Sperm displayed a more polarised DNA methylation distribution to blood; both low (<20%) and high (>80%) levels of methylation were more common.

In comparing the spermatozoal DNA methylome to those of ~6,000 somatic tissue samples, we identified 156,654 CpG sperm-specific hyper- and hypomethylated sites. Six gene ontology terms were enriched among sperm-specific sites, all of which related to transcriptional regulation.

When interrogating DNA methylation covariation between blood and sperm, analyses were restricted to sites meeting minimum variability criteria; range of middle 80% of samples ≥ 5% in blood and sperm. Out of 155,269 such sites, 1% showed a significant correlation of DNA methylation between blood and sperm ($P < 9 \times 10^{-8}$). Using the human dbSNP database, we identified SNPs in the probe sequences of most (>99%) of these correlated sites.

We found no evidence that obesity impacts on DNA methylation covariation between blood and sperm. However, DNA methylation at one site (cg19357369) was significantly associated with obesity status in blood and sperm ($P = 8.95 \times 10^{-8}$).
Limitations, reasons for caution: A small obesity cohort (n = 22) limited our ability to identify modest effects.

Lacking the genetic sequence of our subjects meant we could only speculate as to how SNPs influenced our results, not verify this directly.

The finding of an obesity-associated CpG site in blood and sperm requires replication.
Wider implications of the findings: The lack of DNA methylation covariation between blood and sperm means that blood should not be used as a surrogate tissue for spermatozoa.

Obesity has only a marginal influence on DNA methylation in human spermatozoa.

It is unlikely that DNA methylation is mechanistically involved in intergenerational inheritance of acquired traits.
Trial registration number: Not applicable

O-251 Fallopian tube response to DNA fragmented sperms: the role of Tumor necrosis factor receptors

R. Mohammadi[1], S.O. Mousavi[2], M. Sabbaghian[3], S. Aghajanpour[4], Z. Zandieh[5], T. Maadani[4], R. Aflatoonian[6]

[1]Royan institute for Reproductive Biomedicine., Department of Endocrinology and Female infertility- Reproductive Biomedicine Research Center-, Tehran, Iran ;
[2]Faculty of Advanced Science and Technology- Tehran Medical Sciences- Islamic Azad University, Department of Genetics, Tehran, Iran ;
[3]Reproductive Biomedicine Research Center- Royan institute for Reproductive Biomedicine, Department of Andrology, Tehran, Iran ;
[4]Reproductive Biomedicine Research Center- Royan institute for Reproductive Biomedicine, Department of Endocrinology and Female infertility, Tehran, Iran ;
[5]Iran University of Medical sciences, Department of Anatomical science, Tehran, Iran ;
[6]Reproductive Biomedicine Research Center- Royan institute for Reproductive Biomedicine, Department of Endocrinology and Female infertility-, Tehran, Iran

Study question: Can DNA fragmentation of sperm induce significant changes in immunological response of fallopian tube?

Summary answer: The results show that sperm DNA fragmentation may alter immunological response of fallopian tube.

What is known already: The interaction between the male and female gametes and embryos in the female reproductive system plays an important role in fertility, embryonic development, and implantation. Active immune systems throughout the female genitalia are against viral pathogens and bacterial agents which cause sexually transmitted diseases. TNF receptors super family members are one of the most important variables of immune cells that provide effective host protection. During fertilization sperms carry proteins which are allogenic to the female immune system. Therefore, TNF receptors play an important role in the fallopian tube specially in the presence of sperm.

Study design, size, duration: Fallopian tube epithelial cells were cultured into the tissue culture flasks containing DMEM/F12 with 10% FBS medium. Sperm samples from 20 donors with normal features were collected. The extent of sperm DNA fragmentation was measured by the TUNEL assay. Afterward, samples classified to two groups of normal and abnormal DFI. The third group was fallopian tube cells without sperm.

Participants/materials, setting, methods: Different sperms were co-incubated with fallopian tube cells for 24h. RNA extraction from cells was then fallowed by cDNA synthetized. Finally, PCR array was performed to evaluate TNF receptors genes expression profiling. In addition, this data was validated by q-PCR.

Main results and the role of chance: The results of the data analysis indicated that the expression of some TNF receptors in the vicinity of sperm significantly changes. Sperm DFI was assumed to be effective in different expression of TNF receptors. However, The results has shown that the expression of TNF receptors in cells exposed to abnormal-DFI sperms compared to the cells exposed to sperms with normal DFI had no significant changes. The present study on the effect of spermatozoa on TNF receptors production from the fallopian tube epithelial cells revealed that the expression of cytokines altered between different groups. In conclusion, TNF receptors super family members including TNF, TNFSF10, TNFSF11 and TNFSF11B had the highest expression in the group without spermatozoa.

Limitations, reasons for caution: The major limitation of this study was obtaining semen samples due to ethical and logistical issues. On the other hand, *in vitro* culture system may not directly mimic of *in vivo* environment.

Wider implications of the findings: This study indicate that abnormal DFI can change the expression of cytokines which have essential roles in sperm preservation and fertilization. Therefore, this promising novel outcome might be true that alteration in immunological responses of fallopian tube can disrupt fertilization events.

Trial registration number: not applicable

O-252 piR-32678 in the seminal plasma is a potential predictor for spermatogenic function in testis

T. Wu[1,2,3], L. Li[1,2,3], M. Song[1], C. Chen[1,3], Y. Chen[1,3], Y. Zeng[1,2,3]

[1]Shenzhen Zhongshan Urology Hospital, Fertility Center, Shenzhen, China ;
[2]Shenzhen Zhongshan Urology Hospital, Shenzhen Key Laboratory of Reproductive Immunology for Peri-implantation, Shenzhen, China ;
[3]Shenzhen Zhongshan Urology Hospital, Shenzhen Zhongshan Institute for Reproductive Medicine and Genetics, Shenzhen, China

Study question: Is there any seminal plasma piwi-interacting RNA (piRNA) to predict male fertility?

Summary answer: The expression of piR-32678 in seminal plasma was identified as a noninvasive evaluation of the spermatogenic function in testis.

What is known already: microRNAs (miRNAs) have been reported to be involved in spermatogenesis, and may serve as noninvasive biomarkers for evaluating male fertility. Unlike ubiquitous miRNAs which are secreted from a variety of tissues and organs, piRNAs are mainly expressed in germ cell. In this concern, piRNAs in seminal plasma seems to more reliable to develop as candidate predictor for male fertility.

Study design, size, duration: This retrospective cohort study was conducted from January to April 2018. A total of 31 fertile controls with normal semen parameters, 18 oligospermia, 12 non-obstructive azoospermia (NOA) and 11 obstructive azoospermia (OA) patients were enrolled in this study. Semen analysis was determined according to 2010 WHO reference criteria. Whilst, the seminal plasma was separated from the spermatozoa by high-speed centrifugation.

Participants/materials, setting, methods: Seminal plasma RNAs were extracted and quantified. Samples from 5 cases of NOA and 5 cases of controls were subjected to an Arraystar HG19 4×44K piRNA microarray. Among differential expressed piRNAs, piR-32678 was selected for verification on 26 controls, 18 oligospermia, 7 NOA and 11 OA patients using RT-qPCR. The diagnostic value of piR-32678 for the spermatogenic failure was evaluated by receiver operating characteristic (ROC) curve.

Main results and the role of chance: Firstly, there were totally 3652 piRNAs differentially exhibited in seminal plasma from patients with NOA compared with those from fertile controls (fold change > 2, $P < 0.05$), including 1532 up-regulated piRNAs and 2120 down-regulated piRNAs. Secondly, the qRT-PCR result showed that the relative expression of seminal plasma piR-32678 in oligospermia group [3.22 (2.55, 6.34), $U = 23.50$, $P < 0.001$] and in the NOA group [2.31 (1.65, 3.02), $U = 24.00$, $P = 0.003$] was significantly higher than that in the control group [1 (0.83. 1.42)]. However, no statistically difference was noticed between OA group and the controls [0.92 (0.54, 2.55), $U = 142.00$, $P = 0.973$]. Thirdly, ROC analysis demonstrated that the area under the curve (AUC) of piR-32678 was 0.891 (95% CI: 0.812 - 0.970, $P < 0.001$) for diagnosing spermatogenic failure. When the cut off value of the piR-32678 relative expression was set as 1.53, the sensitivity and specificity were 0.960 and 0.757, respectively.

Limitations, reasons for caution: Small sample size is the first limitation for this study. The findings require to be further validated in a large sample. The biological function of piR-32678 needs to be further investigated in the future study.

Wider implications of the findings: Seminal plasma piRNAs can be considered as new innovative noninvasive molecular makers for evaluating male fertility. Furthermore, piRNAs might provide a new clue for studying the etiology of spermatogenic disorders.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 62: PATHOPHYSIOLOGIC ASPECTS OF IMPLANTATION

08 July 2020 Parallel 4 10:00 - 11:45

O-253 Endometrial gene expression profiling of recurrent implantation failure after in vitro fertilization

İ.G. Albayrak[1,2], F.A. Zarnagh[3], B. Karamustafaoğlu Balcı[4], E. Baştu[5], T. Günel[2]

[1]Uskudar University, Molecular Biology and Genetics, Istanbul, Turkey ;
[2]Istanbul University, Molecular Biology and Genetics, Istanbul, Turkey ;
[3]Istanbul Medical Faculty, Molecular Medicine, Istanbul, Turkey ;
[4]Istanbul Medical Faculty, Department of Obstetrics and Gynaecology, Istanbul, Turkey ;
[5]Acibadem Fulya Hospital, Department of Obstetrics and Gynaecology, Istanbul, Turkey

Study question: Has the expression of endometrial mRNAs in implantation phase of menstrual cycle significantly altered between RIF (Recurrent Implantation Failure) and the control group?

Summary answer: The study results showed that three selected mRNAs were significantly dysregulated in between RIF and control group samples.
What is known already: Successful embryo implantation depends on a well-functioning endometrium as well as a normal healthy embryo. This process might be blocked if either of these variables is defective. Recurrent implantation failure (RIF) is diagnosed when good-quality embryos repeatedly fail to implant after transfer in several in vitro fertilization (IVF) treatment cycles. Expression differences in maternal mRNAs could be reffering to so many diseases including recurrent implantation failure. A better understanding of the underlying mechanisms of RIF will give better treatment opportunities and better outcomes in IVF.
Study design, size, duration: In this study, there are two groups; RIF and normal fertility samples which are collected as endometrial biopsies in implantation phase of menstrual cycle. In the first step, total RNAs isolated from endometrium tissue. Then Droplet Digital PCR (ddPCR) performed for expression analysis of target mRNAs (mRNAs of TNC, WWC1, MME genes) in samples. And bioinformatic analysis of ddPCR results was performed. Study duration lasted from November 2018 until December 2019.
Participants/materials, setting, methods: Endometrial biopsy samples was collected from 35 RIF samples (age <40) and 35 fertile control samples (age <40). Samples were collected in the secretory phase of menstrual cylce (cycle day 20-24). In this study, mRNA expressions of three selected mRNAs were mesuared by ddPCR technique which is a highly sensitive method for measuring expression levels of targeting mRNAs in biological sample.
Main results and the role of chance: The significant feature of this study is the analysis of mRNA expression level in healthy and RIF endometrial biopsy samples which collected in implantation phase. Four target genes (MME, WWC1, TNC) were chosen by PANOGA database regarding to their possible relation with implantation process. Study results showed that, MME and WWC1 genes expression levels are significantly (P<0,05) up-regulated and TNC gene expression level is significantly (P<0,05) down-regulated in RIF samples comparing to the control group.
Limitations, reasons for caution: In this study, one of the challenges was endometrial biopsy step; sample obtaining from normal fertile women was difficult.
Wider implications of the findings: The importance of endometrial gene expression analysis studies lies in the impact of any identification of one or more markers specific for receptivity. In addition to identification of differentially expressed genes, identifying the related pathways is important for uncovering molecular processes in RIF and understanding the mechanisms underlying the disease.
Trial registration number: not applicable

O-254 Prediction of miscarriage in women presenting with threatened miscarriage in their first trimester of pregnancy using biomarkers, ultrasound markers and demographic variables

R. Pillai[1], D. Tincello[1], N. Potdar[1]

[1]University of Leicester, Department of health sciences, Leicester, United Kingdom

Study question: Can we predict miscarriage in women presenting with threatened miscarriage in the first trimester of pregnancy using biomarkers, ultrasound markers and demographic variables?
Summary answer: A log regression model using age, hCG, inhibin A and fetal heart rate can predict miscarriage with a sensitivity of 58% and specificity of 96%.
What is known already: A systematic review and meta-analysis on biomarkers to predict miscarriage has shown ca 125 as the best marker in predicting miscarriage (Pillai *et al*., 2016). Another systematic review conducted on ultrasound markers to predict miscarriage has demonstrated fetal heart rate (FHR) as the most predictive marker for miscarriage with a cut off value of 110 bpm (Pillai *et al.,* 2018). Maternal age is a well-established risk factor for miscarriage. Studies focussing on the combination of markers with FHR has shown promising results. However, existing evidence was limited by the quality of the studies and inconsistencies in the reporting formats.
Study design, size, duration: A prospective cohort study was conducted from December 2015 till September 2017, recruiting 278 women.
Participants/materials, setting, methods: The study included women presenting with bleeding +/- pain into the early pregnancy assessment unit and had a confirmed fetal heartbeat on the scan from 6+0 to 11+6 weeks of gestation. Women with uterine anomalies/ myomas, multiple pregnancies, extrauterine pregnancy and suspected trophoblastic disease were excluded from the study. The study also excluded women who had endometriosis, adnexal masses, exogenous hormones, diabetes, women less than 16 years of age and differently-abled women.
Main results and the role of chance: Out of 278 women recruited, 15 women miscarried (5.34%). Comparison of the miscarried women and those who continued pregnancy in women presenting with threatened miscarriage had shown that the two groups of women were different in the age (P value 0.05) and an objectively assessed bleeding score (P-value 0.03). The two groups were also different in the biomarkers hCG (P-value 0.04), progesterone (P-value 0.03), inhibin A (P value 0.02) and ultrasound markers MGSD (P-value 0.04), CRL (P-value 0.03) and FHR (P-value 0.01). On stepwise multivariate regression analysis, a regression model composed of the variables of age, hCG, inhibin and FHR gave the best sensitivity and specificity to predict miscarriage (P-value 0.0003). The ROC curve for the above regression model showed a sensitivity of 58% and specificity of 96%. The model gave a diagnostic odds ratio (95% CI) of 1.01 (1.01 – 1.02) to predict miscarriage.
Limitations, reasons for caution: Due to fewer numbers of miscarriages, the study was not able to establish a gestation age-specific cut off value for predictive markers. Failure to eliminate those miscarriages due to chromosomal problems might have negatively contributed to the results of the study.
Wider implications of the findings: The proposed prediction model needs to be tested and validated in a prospective research setting with a different study population. Contrary to the results of the previous systematic review, the current study established that ca 125 is not a useful marker to predict miscarriage in women presenting with threatened miscarriage.
Trial registration number: T 15-29

O-255 Does the outcome of fresh embryo transfer affect the outcome of the subsequent frozen-thawed embryo transfer originating from the same cohort of retrieved oocytes

G. Oron[1,2], M. Ronen[1,2], A. Wertheimer[1,2], E. Shlush[1,2], A. Hochberg[1,2], A. Ben-Haroush[1,2], O. Sapir[1,2], Y. Shufaro[1,2]

[1]Beilinson, Infertility and IVF Unit- Helen Schneider Hospital for Women- Rabin Medical Center- Beilinson Hospital, Petach - Tikva, Israel ;
[2]Tel-Aviv University, Sackler Faculty of Medicine-, Tel-Aviv, Israel

Study question: Does the outcome of the fresh transfer affect the subsequent frozen-thawed transfer originating from the same cohort of oocytes
Summary answer: The clinical pregnancy rate of the first frozen-thawed transfer is higher if a clinical pregnancy was attained in the fresh transfer regardless of live birth
What is known already: Numerous factors including maternal and treatment characteristics affect the outcome of an IVF treatment cycle. Current data is indecisive to whether the outcome of a fresh embryo transfer has an impact on the outcome of the subsequent frozen-thawed transfer of embryos originating from the same cohort of retrieved oocytes.
Study design, size, duration: The study included all oocyte collection cycles between January 2009 and September 2019 that had a fresh embryo transfer and at least one frozen-thawed embryo transfer cycle, in a tertiary medical center.
Participants/materials, setting, methods: Frozen-thawed cycles after a fresh cycle with a clinical pregnancy were compared to frozen-thawed cycles after a fresh cycle without a pregnancy. Logistic regression analysis was utilized to adjust for potential confounders including maternal age, treatment protocol, cause of infertility, treatment cycle number, number of oocytes retrieved, number of frozen embryos, day of fresh embryo transfer (cleavage vs. blastocyst) and outcome of the fresh embryo transfer
Main results and the role of chance: During the study period 1,758 oocyte retrieval cycles had a fresh embryo transfer and a subsequent frozen-thawed transfer of embryos originating from the same cohort of retrieved oocytes. The clinical pregnancy rate from the fresh embryo transfers in the study group was 28.9% (508/1758). The clinical pregnancy rate in the first frozen- thawed embryo transfer was 30.1% (153/508) among patients who achieved a pregnancy in the fresh transfer and 23.9% (299/1250) of those who did not (p=0.007). The clinical pregnancy rate from the subsequent frozen-thawed transfer was similar if a live birth was attained from the fresh embryo transfer 28.9% (69/239) or not 25.2% (383/1519); p=0.229. The live birth rate from the subsequent frozen-thawed

transfer was similar if a clinical pregnancy or live birth were attained from the fresh embryo transfer or not. On a multivariate regression analysis, pregnancy in the fresh transfer was a significant independent predictor for a pregnancy in the subsequent frozen-thawed embryo transfer p=0.043. Other significant predictors were maternal age and the treatment protocol.
Limitations, reasons for caution: The limitation of the study is in the retrospective nature of the study
Wider implications of the findings: Identifying predictive factors for the success of frozen-thawed embryo transfers is important to maintain acceptable pregnancy rates while reducing multifetal pregnancies. These can help physicians while counseling patients regarding the number of embryos to transfer taking into consideration the outcome of the previous fresh cycle.
Trial registration number: not applicable

O-256 Conception after early IVF pregnancy loss – Should we wait?

M. Sharon-Weiner[1], H. Gluska[1], S. Farladansky-Gershenabel[1], H. Schreiber[1], A. Wiser[1], A. Shulman[1], A. Hershko-Klement[1]
[1]Meir Medical Center, Obstetrics and Gynecology, Kfar Saba, Israel

Study question: Does the interval between early IVF pregnancy loss and the next IVF cycle predict the cycle's outcome?
Summary answer: Shorter intervals between early IVF pregnancy loss and reinitiating IVF treatment are related to the likelihood of live birth in the subsequent pregnancy.
What is known already: Approximately 5% of IVF cycles end in early pregnancy loss, representing 15% of pregnancies resulting from IVF. There is no consensus regarding the optimal interval between a miscarriage and the next attempt to conceive. Short inter-pregnancy intervals (IPIs) are associated with poor obstetric outcomes in the general population. Despite the abundance of studies examining IPI in the general obstetric population, there is a paucity of studies specifically evaluating IPI in the IVF population.
Study design, size, duration: This retrospective cohort study included 289 women who experienced first trimester IVF pregnancy loss, January 2014–January 2018 and reinitiation of IVF treatment.
Participants/materials, setting, methods: Women who experienced first-trimester pregnancy loss (including chemical pregnancy and miscarriage) following IVF were included. Treatments and follow-up occurred in a tertiary, university-affiliated, medical center. Miscarriages were classified as spontaneous/medical/surgical termination. We calculated intervals between miscarriage and the next cycle, interval to the subsequent pregnancy and number of cycles required**.** Primary outcome measure was the result of the IVF cycle following the miscarriage. Secondary measure was the interval between miscarriage and the next IVF pregnancy.
Main results and the role of chance: Among 289 women diagnosed with first trimester pregnancy loss following an IVF cycle, interval to the subsequent IVF treatment was not associated with the chance of achieving pregnancy. Patients after chemical pregnancy or spontaneous miscarriage experienced shorter intervals to next cycle, as compared with miscarriages managed medically or surgically (Kaplan-Meier survival curve, P=0.01) and higher pregnancy rates (P=0.009).

In a multivariable logistic model, type of miscarriage mattered: compared to chemical pregnancy, odds ratio (OR) for achieving pregnancy after a spontaneous miscarriage was 0.7 (P=0.5); OR=0.3 (P=0.002) for a pregnancy after a medical termination, and OR=0.3 (P=0.001) for a pregnancy following surgical termination. However, the time interval was not a significant factor (P=0.8).

When pregnancy was achieved in the first post-miscarriage cycle, the chance of a live birth was higher with shorter intervals (median 57.5 days), while another miscarriage was significantly related to a longer interval (median 82.5 days) between miscarriage and the next IVF cycle (P=0.03).
Limitations, reasons for caution: This was a non-randomized, retrospective study. Time intervals between cycles was not planned or randomized.
Wider implications of the findings: Following miscarriage, the subsequent IVF cycle should not be postponed, as shorter intervals are associated with greater likelihood of a live birth. We cautiously state that IVF cycles following chemical pregnancy or spontaneous miscarriage, rather than medically or surgically managed miscarriage result in higher pregnancy rates.
Trial registration number: Not applicable

O-257 The effect of vaginal microbiota on the outcome of assisted reproductive technologies

A. Karaer[1], B. Doğan[2], G. Tuncay[3], S. Gunal[4], A.N. Tecellioğlu[5]
[1]Inonu University School of Medicine, Obstetrics and Gynecology, Malatya, Turkey ; [2]School of Engineering- Inonu University-, Department of Biomedical Engineering, Malatya-, Turkey ; [3]Inonu University- School of Medicine, Department of Obstetrics and Gynecology-Division of Reproductive Endocrinology and Infertility-, Malatya, Turkey ; [4]School of Pharmacy- Inonu University, Medical Microbiology, Malatya, Turkey ; [5]Inonu University, Reproductive Sciences & Advanced Bioinformatics Application & Research Center, Malatya, Turkey

Study question: Is there any impact of the vaginal microbiota on the pregnancy rate in women undergoing assisted reproductive techniques (ART)?
Summary answer: Although the pregnancy rate is lower in women with community site type (CST)-IV and CST-V, there was no significant association between CSTs and pregnancy rate.
What is known already: Bacteria in the human body account for 2-3% of total body mass and urogenital tract accounts for around 9% of the total human microbiota. In literature, microbiome studies conducted on female reproductive tract usually investigate the obtained vaginal microbiota data under five different community state types (CSTs). Where, CST-I, II, III and V forms the groups in which *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners,* and *Lactobacillus jensenii* are dominated (relative abundance > 50%), respectively. CST-IV forms the group in which none of the above Lactobacillus species are dominated. Growing evidence demonstrates the importance of vaginal microbiota in human reproductive functions.
Study design, size, duration: A cohort of 223 infertile patients undergoing ART in an university based ART center between May 2016 and May 2019 were enrolled in this study.
Participants/materials, setting, methods: The vaginal swab were collected from posterior vaginal fornix immediately before embryo transfer. Genomic DNA was extracted from vaginal samples and sequenced by the V3-V4 region of 16S ribosomal RNA (rRNA) gene. The vaginal microbiota profiles were assigned to one of the five CSTs based on the dominant bacterial species. The biochemical pregnancy rate (positive hCG test result day 14) was investigated.
Main results and the role of chance: In total the vaginal microbiota of 223 women who underwent a fresh embryo transfer were analyzed. The vaginal microbiata of 123 (55.2%) women dominated by Lactobacillus species, 100 (44.8%) women had bacteria related with bacterial vaginosis predominantly. The overall pregnancy rate was 48.4% (108/223). The pregnancy rate was 53.8% (21/39) in women with CST-I, 66.7% (10/15) in women with CST-II, 50% (32/64) in women with CST-III, 44% (44/100) in women with CST-IV, and 20% (1/5) in women with CST-V. There was no statistically significant difference between CSTs regarding to pregnancy rate. CST-IV was mostly dominated by *Gardnerella vaginalis*, *Streptococcus agalactiae* and *Streptococcus anginosus*. A linear discriminant analysis (LDA) of the vaginal microbiota data concluded that, the Streptococcaceae (family) has the highest contribution (|log10 LDA score| > 4) in the separation of women with positive and negative pregnancy test result.
Limitations, reasons for caution: The major limitation of sequencing are that it does not give information about the viability of the organism and it does not provide information about its biological function. These results are limited to the studied ART population and do not reflect the microbiota profile of a general population.
Wider implications of the findings: Our data demonstrated that the abnormal vaginal microbiota is not associated with poor pregnancy rate for ART treatment.
Trial registration number: Not applicable

O-258 First trimester pregnancy outcomes of subchorionic haematomas in a recurrent pregnancy loss population at a tertiary London teaching hospital.

C. Brooks[1], S. Webb[1], Y. Mulji[1], R. Rai[1]
[1]St Mary's Hospital Imperial College Healthcare NHS Trust, Obstetrics and Gynaecology, London, United Kingdom

Study question: Do subchorionic haematomas increase the likelihood of miscarriage in recurrent pregnancy loss population; are they related to use of aspirin and low molecular heparin?

Summary answer: Subchorionic haematomas do not increase the likelihood of miscarriage in the recurrent pregnancy loss population.
What is known already: There is conflicting evidence on the role of subchorionic haematomas (SCH) on pregnancy outcome and specifically in the recurrent pregnancy loss (RPL) population. Some studies report an association with miscarriage and increase of SCH incidence with the use of Aspirin. This is an important finding to confirm as aspirin and low molecular weight heparin (LMWH) are both established treatment regimens for conditions that can cause recurrent pregnancy loss.
Study design, size, duration: A retrospective observational cohort study on all new patients referred to the early pregnancy clinic at St Mary's Hospital, London, was conducted between January 2017 and June 2019.
Participants/materials, setting, methods: The patients seen in the early pregnancy unit have a history of RPL and are referred by their GP. They are seen in clinic at 6 weeks gestation and have a transvaginal ultrasound scan performed.

Patient data was collected from both Cerner © electronic records and carestream PACsweb imaging.
Main results and the role of chance: In this pilot study of 226 patients the median age of the RPL women were 36 (range 24-45) and had an median number of miscarriages per couple of 4 (range 1-15). There was a miscarriage rate of 28%.
There was a subchorionic haematoma incidence of 17%.
Comparing the RPL patients on medication with those on no medication, there was no increased likelihood of developing a SCH; aspirin alone (OR 1.6 CI 0.55-4.73 p=0.39); LMWH alone (OR 2.1CI 0.66-6.89 p= 0.2) or LMWH and aspirin combined (OR1.3 CI 0.5-3.37 p=0.5).
Women who were on medication for an identified cause of RPL were less likely to miscarry than those who were on no medication. (OR 0.38 CI0.19-0.76 p=0.006). Therefore the likelihood of having a miscarriage is higher in women on no medication where no cause was found for the RPL.
Having a SCH did not increase the likelihood of having a miscarriage in this population (OR 0.65 P 0.31)
Limitations, reasons for caution: It was a retrospective study and therefore relies on accurate record keeping. There will be inter-operator bias, as different sonographers scanned during the clinic.
Wider implications of the findings: In the RPL population we can reassure that having a SCH does not increase the risk of miscarriage in the first trimester. Aspirin and LMWH do not increase the risk of developing a SCH and therefore medication should not be stopped if they develop a SCH.
Trial registration number: Not Applicable

O-259 Low mannose binding lectin level in plasma is a risk factor for recurrent pregnancy loss

C. Nørgaard-Pedersen[1], R. Steffensen[2], O.B. Christiansen[1]

[1]Aalborg University hospital, Obstetrics and gynaecology, Aalborg, Denmark ;
[2]Aalborg University Hospital, Bioanalytic department, Aalborg, Denmark

Study question: Are low or high plasma mannose binding lectin (p-MBL) levels associated with recurrent pregnancy loss (RPL) and the perinatal outcome before and after RPL diagnosis?
Summary answer: While low p-MBL levels are significantly more frequent in RPL patients, high levels are significantly less frequent. No association with adverse perinatal outcomes was found.
What is known already: Low p-MBL levels have been associated with RPL, while relations to high levels have been poorly studied. Reports concerning association between RPL and perinatal outcomes including birth weight and gestational age are conflicting. Some but not all studies suggest an association between low maternal p-MBL levels and reduced birthweight and gestational age, which could be an important factor for the higher frequency of preterm birth and lower birth weight often reported in pregnancies after RPL.
Study design, size, duration: A combined case-control and cohort study in 248 RPL women admitted to the RPL Centre of Western Denmark from January 2016 to December 2019. At first consultation blood samples including p-MBL measurement and perinatal data from previous births were collected. Women were followed until birth or end of study. P-MBL levels were compared to those of 185 female blood donors, while perinatal outcomes were compared to all 3354 singleton births at our hospital in 2017.
Participants/materials, setting, methods: All women had ³3 consecutive pregnancy losses and a regular menstrual cycle, while none had significant chromosomal or uterine abnormalities. Frequencies of different subgroups of p-MBL levels in RPL patients were compared to MBL levels in the control group. Frequencies of adverse perinatal outcomes between subgroups defined by p-MBLlevels were compared. A multivariate analysis was performed identifying risk factors including low p-MBL level for adverse obstetrical outcomes in the patients.
Main results and the role of chance: Significantly more RPL patients had low p-MBL levels (<500 mg/l) (odds ratio [OR] = 2.45, 95% CI 1.61-3.71; p <0.001) and less had high MBL levels (>3000 mg/l) (OR = 0.51; 95% CI 0.33-0.80; p=0.003) compared to controls, while comparing the intermediate p-MBL levels showed no differences (OR = 0.73, 95% CI 0.49-1.07; p = 0.11). A previous moderate (>500 ml) or severe peripartum haemorrhage (>1000 ml) (p<0.001 for both) and previous birth of a boy (p<0.001) were associated with secondary RPL (sRPL). Smoking, increasing age, BMI and number of consecutive miscarriages but not low p-MBL levels were significantly associated with no pregnancy or pregnancy loss after admission. No clear association was observed between maternal p-MBL levels and birthweight or gestational age, neither before nor after RPL.
Limitations, reasons for caution: Only 93 (37.5 %) women gave birth after RPL in the follow-up period, which were too few to find any clear associations between p-MBL levels and adverse perinatal outcomes after RPL.
Wider implications of the findings: While low p-MBL levels are strongly associated with RPL, high levels may play a protective role. A previous large peripartum haemorrhage, previous delivery of a boy and low maternal p-MBL may predispose to a large feto-maternal transfer of fetal cells triggering an abnormal maternal immunization against fetal or trophoblast cells.
Trial registration number: NCT04017754

SELECTED ORAL COMMUNICATIONS
SESSION 63: PROTECTING GAMETE QUALITY

08 July 2020 Parallel 5 10:00 - 11:45

O-260 LH prevents follicular damage and preserves the meiotic potential of oocytes exposed to chemotherapy at the primordial stage

L.M. Castillo[1,2], M.J. Soriano[1], J. Martinez[1,2], A. Pellicer[1,3], S. Herraiz[1]

[1]IVI Foundation - IIS La Fe, Reproductive Medicine, Valencia, Spain ;
[2]University of Valencia, Department of Pediatrics- Obstetrics and Gynecology, Valencia, Spain ;
[3]IVI-RMA Rome, Reproductive Medicine, Rome, Italy

Study question: Does luteinizing hormone (LH) treatment protect follicle viability and meiotic potential of chemotherapy-exposed primordial follicles?
Summary answer: LH improves the meiotic potential of murine metaphase II oocytes (MII-oocytes), avoiding the alkylating agents´ gonadotoxic effects, by the promotion of follicular DNA repair mechanisms.
What is known already: High-dose chemotherapy with alkylating drugs induces detrimental changes on ovaries. Follicle viability is severely affected by DNA damage and apoptosis of oocytes and granulosa cells (GCs), leading to impaired follicular development and depletion. Early activation of DNA repair mechanisms, like homologous recombination through the ataxia-telangiectasia-mutated (ATM) pathway, is crucial for cell survival after cytotoxic events. Previous results suggested LH treatment as an alternative for fertility preservation based on its protective role on the ovarian reserve against chemotherapy. Therefore, we aimed to assess the follicular protective mechanisms of LH and the meiotic potential of chemotherapy-exposed MII-oocytes in a mouse model.
Study design, size, duration: Experimental study where twenty-four 7-week old CD-1 female mice were exposed to three experimental conditions (n=8/group): Control, chemotherapy (ChT) and ChT+LH. The ChT-treated groups were intraperitoneally injected with 12mg/Kg-busulfan and 120mg/kg-cyclophosphamide. The LH-treated animals received a pre-treatment dose with 1IU,

24 hours before ChT, followed by a second 1IU-dose administered with chemotherapy. Control-mice received saline. Ovaries from 6 animals/group were collected at 12 and 24 hours, while the remaining mice were maintained for 30 days.

Participants/materials, setting, methods: The ATM-pathway, by Rad51 gene expression, was evaluated by RT-qPCR, while apoptotic (cleaved caspase-3) and anti-apoptotic (Bcl2) proteins were quantified by western-blot, on ovarian samples at 12 and 24h. Additionally, the 12h samples were screened for follicle DNA damage and apoptosis by γH2AX-staining and TUNEL-assay, respectively. The remaining animals were superovulated (10IU-PMSG + 10IU-hCG, 18 hours later) for MII-oocyte collection. Thus, spindle formation and chromosome disposition, referred to equatorial plate, were analyzed by confocal microscopy.

Main results and the role of chance: LH treatment increased the expression of the DNA repair gene Rad51 at the 12h (Control: 1, ChT: 2.0±0.5; ChT+LH: 2.7±1.3; p=0.020 and p=0.019, respectively) and 24h timepoints (Control: 1, ChT: 0.5±0.5, ChT+LH: 1.4±0.3). The activation of the DNA repair signalling led to a rise of Bcl2/cleaved caspase-3 protein ratio, decreased by chemotherapy, enhancing cell survival on 24h-ovaries (Control: 2.5±0.9, ChT: 0.8±0.3, ChT+LH: 2.0±1.3).

Furthermore, LH treatment reduced the significant increase in the number of γH2AX-positive oocytes induced by chemotherapy (Control: 19.6±0.8%, ChT: 64.6±3.1%, ChT+LH: 42.6±4.1%; p=0.034 and p=0.021, respectively). Moreover, these positive effects were also observed in the GC integrity, by reducing the amount of follicles with >20% of TUNEL-positive GCs (Control: 1.6±0.8%, ChT: 8.5±0.8%, ChT+LH: 5.1±0.9%; p=0.034 and p=0.043), during the first 12 hours after treatment.

The meiotic potential, referring to MII-oocytes derived from follicles at the primordial stage during chemotherapy administration, was seriously affected in the ChT group, with a 34.5% decrease in spindle area (Control: 127.7±20.5μm^2, ChT: 83.7±7.7μm^2; p=0.019). Nevertheless, LH treatment was able to avoid this effect, preserving control-like values (ChT+LH: 119.7±2.7μm^2; p=0.034). Furthermore, LH diminished the number of MII-oocytes with at least one misaligned chromosome compared to ChT group (Control: 12.5%, ChT: 83.3%, ChT+LHx1: 58.3%).

Limitations, reasons for caution: Although these findings represent the first steps of a new strategy for fertility preservation in cancer patients, this is an animal model study developed in mouse ovarian samples. Therefore, these results should be validated in order to properly identify the repairing mechanisms in a preclinical approach with human samples.

Wider implications of the findings: LH treatment minimizes the deleterious effects induced by alkylating agents on follicular viability. The enhancement of DNA repair systems seems to be one of the main protective mechanisms promoted by LH. This improvement would contribute to produce MII-oocytes with increased potential to properly complete the meiosis II.

Trial registration number: not applicable

O-261 The developmental capacity of In Vitro Matured Oocytes originating from cumulus oocyte complexes (COCs) found during oVArian tissues (OT) preparation is compromised

K. Tilleman[1], S. Lierman[2], A. Tolpe[3], I. De Croo[4], S. De Gheselle[5], J. Defreyne[6], G. T'Sjoen[6], M. Baetens[7], A. Dheedene[7], B. Menten[7], P. De Sutter[8]

[1]Ghent University Hospital, Department for Reproductive Medicine- Ghent Fertility And Stem cell Team G-FAST, Gent, Belgium ;
[2]Ghent university Hospital, Department for Reproductive Medicine- Ghent Fertility And Stem cell Team G-FAST, Gent, Belgium ;
[3]Ghent University Hospital, Department for Reproductive Medicine- Ghent Fertility And Stem cell Team G-FAST, Ghent, Belgium ;
[4]Ghent University Hospital, Department for Reproductive Medicine- Ghent Fertility And Stem cell Team G-FAST, Ghent, Belgium ;
[5]Ghent University Hospital, Department for Reproductive Medicine- Ghent Fertility And Stem cell Team G-FAST, Ghent, Belgium ;
[6]Ghent University Hospital, Department of Endocrinology- Center for Sexology and Gender, Ghent, Belgium ;
[7]Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium ;
[8]Ghent University Hospital, Department for Reproductive Medicine- Ghent Fertility And Stem cell Team G-FAST, Ghent, Belgium

Study question: What is de fertilization capacity of **I**n **Vi**tro **M**atured **O**ocytes found during o**VA**rian tissues preparation(IViMOVA), subsequent embryo development and are these embryos euploid or do they harbor chromosomal abnormalities?

Summary answer: IViMOVA oocytes show lower fertilization rates, impaired embryo development characterized by irregular cleavage patterns and early embryo arrest apparently not related to chromosomal abnormalities.

What is known already: The collection of COCs during preparation of OT has been described as the maximization of fertility preservation. This technique is sometimes mistaken with IVM in Polycystic Ovary Syndrome(PCOS) patients where mild stimulation or primed ovarian punction show maturation rates of 70% and successful treatment outcomes. On the contrary, IViMOVA studies in literature show an average maturation rate of around 30% and the description of the developmental capacity of these oocytes is largely absent. IViMOVA reports in literature are mostly case reports and to our knowledge, so far 3 live births have been described (Segers I.,2015, Uzelac P.,2015, Prasath EB.,2014).

Study design, size, duration: During OT preparation, COCs were collected from surplus medulla, in vitro matured (MediCult IVM medium,Origio) for 44-48h and MII were vitrified(Irvine Scientific). Warmed intact MII were injected after 2h and cultured in sequential medium(Cook) in the Embryoscope®(Vitrolife). For genetic analysis, cells were lysed and DNA was amplified using the Sureplex Amplification system(Illumina), followed by shallow whole genome sequencing (sWGS) (average genome coverage of 0.1-1x), for the detection of aneuploidy or subchromosomal aberrations (resolution ~5Mb).

Participants/materials, setting, methods: From Nov.2015-Jan.2019, in total 93 persons (median age 20.7y) underwent hysterectomy with bilateral oophorectomy in the context of gender affirming surgery after a median duration of testosterone treatment of 75.3weeks. At the time of hysterectomy serum was analyzed for AMH-FSH-LH-E2-Progesterone-Testosterone-SHBG. A negative binomial regression model for counts was applied (SPSS) to find biochemical parameters associated with MII vitrified. Frozen thawed sperm form 1 donor (30y, proven fertile) was used for all ICSI procedures.

Main results and the role of chance: For the developmental capacity study, 1765 COCs of 80 patients were in vitro matured in 5%CO_2, 6%O_2 at 37°C which resulted in 390MII oocytes (22%maturation rate) being vitrified. After correction of demographic variables (duration of testosterone treatment, age and BMI), AMH was significantly (P=0.0058) associated with the number of MII vitrified. 238MII oocytes were donated for this study of which 208 were warmed and 151 (72.6%) intact. ICSI was performed in 92%(139/151) of the intact warmed oocytes. After ICSI, 35% of the oocytes showed normal fertilisation (48/139); 12%(17/139) were 1pn, 19%(27/139) were 0pn and 15%(21/139) were 3pn or more. After ICSI, 19%(26/139) were degenerated. Only 2pn were further cultured and embryo development was monitored. On day 3, 52% of the 2pn (25/48) developed up the cleavage stage of which 44%(11/25) were 6 cell or more. Out of 139 injected oocytes and 48 normally fertilized, only 1 (2%) blastocyst (4BB) was obtained on day 5. Timelapse imaging showed direct or reversed cleavage, no extrusion of the 2nd polar body and arrest in the cleavage stage. For genetic analysis, 32 embryos were available and was successful in 30/32 embryos. Interpretable results showed normal chromosomal patterns in 43% (13/30) of the embryos.

Limitations, reasons for caution: This descriptive cohort study used tissue from transgender patients undergoing men with testosterone treatment, hence it is possible that this could affect the developmental capacity of the oocytes although previous work from our group has shown that the oocytes look morphologically normal and display normal spindles (Lierman S., 2017).

Wider implications of the findings: IViMOVA should be considered an innovative technique (Provoost V. 2014). As this technique is often described as a maximization of fertility preservation for oncology patients, information should be given on the uncertainty concerning the developmental capacities of these oocytes and the lack of data in scientific literature.

Trial registration number: This study was funded by FWO TBM T001016N and was approved by the Ghent University Hospital Ethical Committee (UZ Ghent Reference: 2015/0124, Belgian registration number B670201523543) and the injection of the in vitro matured oocytes and creation of embryos for this study was approved by the Belgian Federal Ethics Committee (reference Adv_056).

O-262 Effects of cancer on biology of granulosa cells, follicular fluid and quality of oocytes

G. Younes[1], W. Buckett[1], Q. Yang[2], W.Y. Son[1], A. Volodarsky-perel[1], T. Tulandi[1], H.J. Clarke[2]

[1]Mcgill University Health Centre, reproduction centre, montreal, Canada ; [2]Mcgill University Health Centre, research institute, montreal, Canada

Study question: Do factors associated with cancer damage the granulosa cells of the ovarian follicle or alter the follicular fluid, leading to abnormal development of the egg?

Summary answer: Inhibitors of Wnt signaling pathway and regulators of cell growth and differentiation, cell-cell interactions and adhesion are downregulated in cancer patients.

What is known already: The adverse effects of chemotherapy and radiotherapy on fertility are well known. Cryopreservation of oocytes and embryos are considered the standard of care for preserving fertility in reproductive-age cancer patients before starting treatments.

Whether the malignancy itself has a negative effect on the reproductive system remains unclear. Some clinical studies have shown apparent adverse effects of malignant disease on the response to ovarian stimulation and on the quality and performance of oocytes, whereas others have shown no difference when compared to healthy controls.

Study design, size, duration: This prospective pilot study included 20 patients treated at our academic reproductive centre. Patients could be divided into two groups: a study group that consisted of 10 cancer patients treated for fertility preservation before chemotherapy or radiotherapy and a control group of age-matched 10 healthy fertile patients treated with IVF due to male factor or social fertility preservation. Research and Ethics Board of McGill University Health Centre approved the study.

Participants/materials, setting, methods: Cumulus cells and follicular fluid were collected from all patients. Total RNA of ten samples was extracted from the cumulus cells. We evaluated mRNA expression profiles of cumulus cells using next generation sequencing by Illumina Novaseq 6000. The follicular fluid of all twenty samples was tested by automatic immunoassay for C -reactive protein (CRP) as an inflammatory marker, cortisol as a stress marker, and insulin like growth factor.

Main results and the role of chance: All patients were treated with the antagonist protocol. The number of total oocytes retrieved, and the number of mature or immature oocytes in the 2 groups were comparable. Three samples from the study group and three others from the control group were further analyzed by mRNA sequencing. Compared with controls, 85 known mRNAs were differentially expressed in cancer patients' cumulus cells, including 45 mRNAs upregulated and 40 mRNAs downregulated. The potential regulating roles of mRNA and gene annotation showed downregulation of genes that act as regulators of cell growth and differentiation, cell-cell interaction and adhesion (*GPC4, ID3, CGC20, IGSF-9, CPXM1*). We also found that two strongly downregulated genes are inhibitors of the canonical Wnt signaling pathway (*SFRP4*, *WIF1*). The concentrations of CRP, cortisol, or insulin like growth factor in the two groups were comparable.

Limitations, reasons for caution: This pilot study has a small sample size. Two patients were treated with letrozole which may have an effect on the results.

Wider implications of the findings: Our data suggest that cellular regulators and inhibitors of the Wnt signaling pathway may be downregulated in cancer.

These changes may have negative effects on cumulus oocyte expansion, ovulation, and luteinization.

Our results provide novel candidates for molecular targets in the research of the effect of cancer on female fertility.

Trial registration number: none

O-263 Pre-treatment with Granulocyte-Colony Stimulating Factor does not protect germ cell populations in the immature human testis from Cisplatin-induced germ cell loss

G. Matilionyte[1], R.A. Anderson[1], R.T. Mitchell[1]

[1]The University of Edinburgh, Centre for Reproductive Health, Edinburgh, United Kingdom

Study question: Does Cisplatin affect germ cell populations in the immature testis and can this effect be rescued by treatment with Granulocyte-Colony Stimulating Factor (GCSF)?

Summary answer: Cisplatin exposure causes acute loss of germ cells in the immature human testis and this effect was not rescued by short-term exposure to GCSF.

What is known already: Long-term survival rates for children with cancer are more than 80%. However, childhood cancer treatment may result in subsequent infertility. Cisplatin is one of the most commonly used drugs for childhood cancers. Knowledge of the effects of Cisplatin exposure in childhood is still limited. Moreover, there are currently no clinical agents to protect spermatogonial stem cells from chemotherapy-induced damage. Animal studies suggest that GCSF may protect germ cells in males and females, however, there is no information whether GCSF could prevent loss of germ cells in human testes.

Study design, size, duration: *In-vitro* culture of human fetal testis tissues was utilised as an established model for pre-pubertal testis development. Tissues were exposed to: 1) 0.5 µg/ml Cisplatin or vehicle control on day 4 for 24hrs and kept in culture until 72 and 240 hours post-exposure; 2) combined treatment of Cisplatin with GCSF (5 or 20 ng/ml) for first 7 days (3 days prior, during and 3 days after Cisplatin exposure).

Participants/materials, setting, methods: Second trimester human fetal testis tissue fragments (14-22 gestational weeks; n=3-14) were cultured in a 'hanging drop' system. Tissue was cultured for 7 and 14 days in total which reflected 72 and 240 hours post-exposure to Cisplatin.

Quantification of germ cell populations (cells per tubular area (mm^2)) was performed on sections stained for AP2γ (gonocytes) and MAGE-A4 (pre-spermatogonia). Statistical analysis was performed using two-way ANOVA to account for inter-individual variation between fetuses.

Main results and the role of chance: Cisplatin exposure significantly reduced the number of gonocytes (478.7±85.30 vs 379.7±92.29 cells/mm^2; $p<0.01$) at 72 hours post-exposure compared with vehicle-exposed controls, whereas the number of pre-spermatogonia was unchanged. After 240 hours, exposure to Cisplatin resulted in significant reduction in both gonocyte (327.1±45.13 vs 193.5±37.94 cells/mm^2; <0.0001) and pre-spermatogonial (393.0±36.73 vs 324.0±60.45 cells/mm^2; $p<0.01$) numbers.

In combined treatment experiments, addition of GCSF from 3 days prior to 3 days after Cisplatin exposure did not protect against the reduction of germ cell numbers 72 or 240 hours post-Cisplatin exposure.

Limitations, reasons for caution: Human pre-pubertal testis tissue is of limited availability, thus, a validated *in-vitro* system using human fetal testis was utilised. These tissues contain similar germ cell sub-populations, which includes gonocytes (present in infancy) and pre-spermatogonia (present throughout pre-puberty). 'Hanging drop' culture might not recapitulate all *in-vivo* aspects of immature testis microenvironment.

Wider implications of the findings: This study shows that Cisplatin exposure causes early loss of gonocytes in the immature human testis, whereas the effect on (pre)spermatogonial number is delayed. This germ cell reduction could not be protected by concurrent GCSF treatment. Alternative regimens of GCSF supplementation are currently under investigation and will be discussed.

Trial registration number: Not applicable

O-264 In vitro fragmentation of ovarian tissue activates primordial follicles through the Hippo pathway

C. De Roo[1], S. Lierman[1], K. Tilleman[1], P. De Sutter[1]

[1]University Hospital Ghent UZ, Reproductive Medicine- Department of Gynaecology, Ghent, Belgium

Study question: What is the role of the Hippo and PI3K/Akt pathway in follicles during ovarian tissue culture in tissue derived from oncological patients and transgender men?

Summary answer: Results highlight a Hippo pathway driven primordial follicle activation *in vitro*, predominantly from day 0 to day 4

What is known already: *In vitro* OT culture aims at activating and maturing primordial follicles for fertility restoration in patients with a threatened ovarian reserve. Not all patients are eligible for ovarian cortex transplantation and therefore several groups attempt to culture ovarian tissue in vitro.

Cortex fragmentation disrupts the Hippo pathway, leading to increased expression of downstream growth factors and follicle growth. The PI3K/Akt pathway is considered the intracellular pathway to where different extracellular factors involved in primordial follicle activation *in vivo* converge to. In order to optimize current ovarian tissue culture models, information on progression of these pathways during tissue culture is mandatory.

Study design, size, duration: The first step of a multistep cortex culture system was performed using 144 ovarian cortex pieces of in total 6 patients. Per patient, 24 cortical strips were cultured for 6 days and 6 pieces per patient were collected for downstream analysis of follicle development and Hippo and PI3K/Akt pathway targets every second day.

Participants/materials, setting, methods: OT was obtained from oncological (N=3; 28.67+/-4.51years) and transgender (N=3; 23.33+/-1.53 ears) patients. Follicles were analysed using hematoxylin-eosin staining and pathways were studied using immunohistochemistry and precise follicle excision by laser capture microdissection for RT-qPCR analysis. MIQE guidelines for RT-qPCR were pursued. Reference gene selection (GAPDH, RPL3A, 18s rRNA) was performed using GeNorm Reference Gene Selection Kit. Statistical analysis was conducted with IBM SPSS Statistics 23 (Poisson regression, negative binomial regression, ANOVA and paired t-test).

Main results and the role of chance: Immunohistochemical analysis confirmed a Hippo pathway driven primordial follicle activation by mechanical manipulation of the cortical strips. Ovarian tissue preparation and culture induced the inhibitory pYAP to disappear in granulosa cells of primordial follicles on day 2. The stimulatory YAP on the contrary appeared in primordial granulosa cells over increasing culture days. Looking at the YAP target CTGF, a significantly up regulated CTGF was noted in primordial follicles when comparing day 2 and day 4 (ratio day 2/4 = 0.082; $p<0.05$), clearly showing an effect on the Hippo pathway in primordial follicles during tissue culture.

Follicle classification objectified a significant drop in estimated primordial follicle counts in the oncological cohort (-78%; p=0.021) on day 2 and in transgender cohort on day 4 (-634%; p=0.008). Intermediate follicle counts showed a non-significant trend to increase during culture and this follicle recruitment and growth resulted in a significant raise in estimated primary follicle counts on day 6 in oncological patients (170%; p=0.025) and although limited in absolute numbers, a significant increase in secondary follicles on day 4 (367%; p=0.021) in the transgender cohort. Subsequent antral follicle development could not be observed.

Limitations, reasons for caution: A limitation is the small sample size, inherent to this study subject, especially as a large amount of tissue was needed per patient to reduce inter patient variation in different downstream analysis techniques. A particular and specific weakness of this study is the inability to include an age-matched control group.

Wider implications of the findings: These findings support an adapted tissue preparation for Hippo pathway disruption and a shorter first phase of tissue culture. This work may also have a reflection on transplantation of cryopreserved tissue as larger strips (and thus slower burnout because of less Hippo pathway disruption) could be a benefit.

Trial registration number: This research was financially supported by the Foundation Against Cancer (Stichting tegen Kanker) and Flemish Foundation of Scientific Research (FWO Vlaanderen), Belgium

O-265 Purging human ovarian cortex of contaminating leukaemic cells by targeting the Mitotic Catastrophe Signalling Pathway

L. Eijkenboom[1], C.L. Mulder[2], B.A. Van der Reijden[3], N.M. Van Mello[4], J. Van Leersum[1], D.D.M. Braat[1], C.C.M. Beerendonk[1], R. Peek[1]

[1]Radboudumc, Obstetrics and Gynaecology, Nijmegen, The Netherlands ; [2]Amsterdam UMC, Reproductive Biology, Amsterdam, The Netherlands ; [3]Radboudumc, Laboratory Medicine, Nijmegen, The Netherlands ; [4]Amsterdam UMC, Obstetrics and Gynaecology, Amsterdam, The Netherlands

Study question: Is it possible to eliminate metastasized chronic myeloid leukaemia (CML) and acute myeloid leukaemia (AML) from ovarian cortex by inhibition of Aurora B/C kinases (AURKB/C)?

Summary answer: Inhibition of AURKB/C *ex vivo* effectively eradicated experimentally induced CML and AML tumour foci and viability of ovarian tissue was not compromised by this treatment.

What is known already: Despite the success of ovarian tissue cryopreservation, autotransplantation is not without risk since malignant cells may be present in the graft. Safety procedures to detect minimal disseminated disease render the fragment useless for autotransplantation and remaining fragments could still harbour malignant cells. Strategies to separate follicles from possibly contaminated stromal cell compartment followed by *in vitro* maturation or grafting to an artificial ovary are actively pursued, but are experimental. We have recently shown complete elimination of rhabdomyosarcoma cells by efficient *ex vivo* purging of ovarian cortex fragments is possible, allowing treatment of all cortex fragments without compromising ovarian tissue integrity.

Study design, size, duration: Human ovarian cortex tissue with experimentally induced tumour foci of CML and AML were exposed to a 24 h treatment with an AURKB/C inhibitor to eliminate malignant cells by invoking mitotic catastrophe. After treatment the inhibitor was removed followed by an additional culture period of 6 days to allow any remaining tumour cells to form new foci. Ovarian tissue integrity was analysed by 4 different assays. Appropriate controls were included in all experiments.

Participants/materials, setting, methods: Tumour foci of CML and AML cell lines were induced in ovarian cortex from transgender men undergoing oophorectomy. Presence of any remaining cancer cells after *ex vivo* treatment was analysed by (immuno)histochemistry of serial sections from entire tissue fragments. Effect of the AURKB/C inhibitor exposure on the viability of ovarian cortex tissue and follicles was determined by histology, glucose uptake assay, follicular viability assay and an assay for the *in vitro* growth of small follicles.

Main results and the role of chance: Foci of metastasized CML and AML cells in ovarian cortex tissue were severely affected by a 24 h *ex vivo* treatment with an AURKB/C inhibitor, leading to the formation of multi-nuclear syncytia and large scale apoptosis. Only the megakaryocytic CML cell line MEG-01, known for its aberrant AURKB expression, was not affected. Ovarian tissue morphology and viability was not compromised by the treatment, as no significant difference was observed regarding the percentage of morphologically normal follicles, follicular viability, glucose uptake or *in vitro* growth of small follicles between the ovarian cortex treated with the AURKB/C inhibitor and the control.

Limitations, reasons for caution: The persistence of CML and AML metabolically-active multinuclear syncytia after treatment precludes the use of molecular techniques to verify the complete absence of viable tumour cells. The functional integrity of the ovarian cortex tissue after *ex vivo* treatment requires further investigation *in vivo*.

Wider implications of the findings: Purging of CML/AML metastases in ovarian cortex is possible by targeting the Mitotic Catastrophe Signalling Pathway using an AURKB/C inhibitor without harming ovarian tissue. This provides a therapeutic strategy to prevent reintroduction of leukaemia and enhances safety of autotransplantation in leukaemia patients currently considered at high risk for ovarian involvement.

Trial registration number: not applicable

O-266 The role of F-actin and MYO10 distribution in quality assessment of fresh and frozen-thawed human growing follicles

S. Granados Aparici[1], T. Tulandi[1], W. Buckett[1], W.Y. Son[1], G. Younes[1], J.T. Chung[1], S. Jin[1], H. Clarke[1], A. Volodarsky-Perel[1]

[1]McGill University Health Centre, Obstetrics and Gynecology, Montreal, Canada

Study question: Does the distribution of F-actin and MYO10 protein differ in human preantral follicles from fresh and frozen human ovarian tissue?

Summary answer: Cryopreservation and the freezing method have an impact on the distribution pattern of F-actin and MYO10 protein in granulosa cells and oocytes.

What is known already: Cryopreservation of ovarian tissue has emerged as an option for fertility preservation for oncological patients. Slow freezing and vitrification, the two major methods of ovarian tissue cryopreservation, may disturb essential communication between oocyte and granulosa cells. Granulosa cells communicate with the oocyte via specialized actin-rich filopodia termed transzonal projections (TZPs) that enable the oocyte to be supplied with key nutrients and regulatory signals. MYO10 protein regulates filopodial growth and/or function and may work similarly in TZPs. However, the effects of freeze-thawing on the expression and distribution of MYO10 and F-actin in TZPs remain unclear.

Study design, size, duration: Frozen ovarian tissue was donated by patients who underwent ovarian tissue cryopreservation due to malignant disease. Fresh ovarian tissue was prospectively collected from patients who underwent ovarian surgery during the period from 2017 to 2019. Only patients with normal ovaries were included in this analysis. Ovarian follicles at primordial to secondary stages were retrieved from patients who donated fresh ovarian tissue (n=4), underwent vitrification (n=3), and slow freezing (n=5) of ovarian tissue.

Participants/materials, setting, methods: Fresh and thawed samples were immediately delivered for follicle harvesting. Follicles comprising a morphologically normal oocyte and non-pyknotic granulosa cells were stained using phalloidin and anti-MYO10. Confocal microscopy was used to image equatorial optical sections of each follicle. Parameters measured were the number of MYO10 foci and intensity of F-actin in the granulosa cells and oocytes. The correlation between the oocyte diameter and the structures associated with TZP development (MYO10 foci and F-actin) was evaluated.
Main results and the role of chance: A total of 180 good-quality follicles with a mean oocyte diameter of 36.6±9.1 µm from women of 22-37 years of age were included in the analysis. A positive correlation between oocyte diameter and MYO10 distribution in granulosa cells (correlation estimate (CE) 0.63; 95% confidence interval (95%CI) 0.43-0.77; P=0.001) and in oocytes (CE 0.43; 95%CI 0.19-0.63; P=0.001) was demonstrated in fresh follicles. In frozen-thawed follicles, a correlation was observed for MYO10 distribution in granulosa cells (CE 0.22; 95%CI 0.04-0.38; P=0.02) but not in oocytes. When vitrified follicles were compared to those subjected to slow freezing, the correlation between oocyte diameter and MYO10 distribution in granulosa cells was revealed only in the slow freezing group (CE 0.38; 95%CI 0.17-0.55; P=0.0005). Strikingly, large MYO10 aggregates within the oocyte were significantly more common after slow freezing compared to vitrification [25(30.5%) vs. 4(9.1%); P=0.007]. Correlation between oocyte diameter and F-actin intensity was observed in the slow freezing group (CE 0.31; 95%CI 0.09-0.49; P=0.006) but not in the vitrified follicles. The different patterns of MYO10 and F-actin distribution in growing fresh and thawed follicles suggests a significant impact of cryopreservation and method of freezing on the structures mediating physical communication between oocyte and granulosa cells.
Limitations, reasons for caution: Only follicles from patients without ovarian pathology were included. The effect of ovarian pathology on the structures associated with TZP development in fresh and frozen-thawed ovarian tissue is a matter for further studies.
Wider implications of the findings: Our study suggests that MYO10 distribution and F-actin intensity in granulosa cells as well as the presence of MYO10 aggregates in oocytes can be used for quality assessment of growing follicles in fresh and frozen-thawed ovarian tissue.
Trial registration number: Not applicable

O-267 Protection of the mouse testis tissue and sperm production from X-ray induced damages by pharmaceutical compounds that increase telomerase

H. Elul[1], **E. Priel**[1]

[1]*Ben-Gurion University of the Negev, The Shraga Segal Dept. Immunology-Microbiology & Genetics, Beer-Sheva, Israel*

Study question: Do pharmaceutical compounds (AGS) that increase telomerase protect the mouse testes from damages induced by X-ray?
Summary answer: A single AGS treatment increased telomerase in the mouse testes and protected the testis and sperm production from the damages induced by X-ray radiation.
What is known already: The telomerase reverse transcriptase (TERT) is expressed and active in the testes and is important for the spermatogenesis process. Short telomeres were identified in men with oligozoospermia and idiopathic infertility. Shortened telomeres in spermatozoa are markers for abnormal spermatogenesis. In addition to its role in the re-elongation of telomeres, TERT possesses non-canonical functions: protects cells from apoptosis, participates in the repair of DNA strand breaks and regulates the expression of genes. We synthesized novel compounds that transiently increased TERT expression and activity in various human and mouse cells and tissues. The compounds protected cells from damages induced by oxidative stress.
Study design, size, duration: Adult mice were divided to various treatment groups (>10 mice per group): with/ without exposure to X-ray radiation and with/without AGS treatment or vehicle treatment. At various intervals, post irradiation and treatment, the mice were sacrificed and their testes including the epididymis were removed and subjected to further analysis.
Participants/materials, setting, methods: Testis slices were stained with hematoxylin- eosin for tissue morphology. To identify the various cell types in the seminiferous tubules we used specific markers (VASA, CREM, acrosin) applying the immunohistochemistry procedures. The expression of TERT and specific spermatogenesis genes was measured by qRT-PCR. The presence of DNA strand breaks was detected by gamma-H2AX and cell proliferation by PCNA. Sperm cells were isolated from the epididymis, counted and analyzed for motility and morphology.
Main results and the role of chance: TERT expression and activity in the mouse testis increased following a single AGS dose. The expression of spermatogenesis markers (VASA and CREM) increased followed by a significant enhancement in the sperm count. Exposure of mice to X-ray radiation (2.5 Gy) damaged the testis tissue and decreased sperm count and sperm parameters (motility, normal morphology). Treatment of the X-ray irradiated mice with a single dose of AGS, significantly restored testis tissue morphology, the expression of spermatogenesis markers and decreased the number of seminiferous tubules that exhibited DNA strand breaks. AGS treatment of irradiated mice restored the sperm count and sperm motility and reduced the percentage of sperm cells with altered morphology. A higher sperm count and an increase in testis tissue regeneration, following X- ray radiation, was also detected in AGS treated mice 21 and 30 days after irradiation and treatment.
Limitations, reasons for caution: We could not use an X- ray dose that completely abolished sperm production. Therefore, under these conditions no significant differences in the ability to produce offspring, between X-ray irradiated and X- ray irradiated + AGS treated mice, were observed
Wider implications of the findings: This study suggests the ability of telomerase increasing compounds to restore the normal tissue morphology, the spermatogenesis process and the sperm count in damaged testes. Therefore, these compounds may be used as possible therapy in conditions that reduced male fertility.
Trial registration number: NA

SELECTED ORAL COMMUNICATIONS

SESSION 64: PROSPECTIVE CARRIER SCREENING OF ART COUPLES

08 July 2020 Parallel 6 10:00 - 11:45

O-268 Insights from the largest study on the genetics of sporadic and recurrent miscarriage

T. Laisk[1]

[1]*Institute of Genomics- University of Tartu, Estonian Genome Center, Tartu, Estonia*

Study question: Can we discover and map the maternal genetic susceptibility and underlying biology of sporadic and recurrent miscarriage using large-scale biobank data?
Summary answer: Our results confirm miscarriage as a complex partly heritable phenotype and implicate novel biology through regulation of genes involved in placental biology.
What is known already: Miscarriage is a common complex trait that affects 10-25% of all clinically confirmed pregnancies. Miscarriage is associated with excessive bleeding, infection, depression, infertility and an increased lifetime risk of cardiovascular disease. A variety of predisposing factors have been associated with increased miscarriage risk, and it has a genetic component that thus far has remained poorly characterized.
Study design, size, duration: Genome wide association study (GWAS) meta-analysis of up to 69,118 sporadic miscarriage cases from seven different ancestries, 750 recurrent miscarriage cases of European ancestry, and up to 359,469 female controls from biobanks all over the world.
Participants/materials, setting, methods: We investigated the genetic architecture of miscarriage with biobank-scale GWAS, Mendelian randomization, heritability, genetic correlation and functional annotation analyses.
Main results and the role of chance: We identify one genome-wide significant association (rs146350366, minor allele frequency (MAF) 1.2%, P=3.2×10-8, odds ratio (OR)=1.4) for sporadic miscarriage in our European ancestry meta-analysis and three genome-wide significant associations for recurrent miscarriage (rs7859844, MAF=6.4%, P=1.3×10-8, OR=1.7; rs143445068, MAF=0.8%, P=5.2×10-9, OR=3.4; rs183453668, MAF=0.5%, P=2.8×10-8, OR=3.8). Following functional annotation linked these associations with genes

related to placental biology. We found a heritability of 29% (95%CI 20%-38%) for miscarriage. Consistent with observational associations, we found significant genetic correlations between sporadic miscarriage and number of children ($p=7.210^{-9}$). The Mendelian randomization analyses suggest that smoking may causally increase the risk of sporadic miscarriage. Finally, our analysis of health outcomes associated with miscarriage confirms previously observed observations and identifies several novel ones.

Limitations, reasons for caution: Only maternal genetic data was analysed. Further studies are needed to assess the functional impact of the associated variants.

Wider implications of the findings: Our study shows the potential involvement of genes regulating placental function in the etiopathogenesis of miscarriage and highlights the utility of large population-based biobank data for understanding these understudied pregnancy complications. This research is presented on behalf of the International Miscarriage Genetics Consortium.

Trial registration number: NA

O-269 High detection rates in both IVF and general population confirm the clinical utility of Expanded Carrier Screening for the management of reproductive genetic risk

M. Fabiani[1], S. Caroselli[1], L. Girardi[1], C. Patassini[1], M. Poli[1], C. Simon[1,2,3,4], A. Capalbo[1,2]

[1]Igenomix Italia, Reproductive Genetics, Marostica VI, Italy ;
[2]Igenomix Foundation, Reproductive Genetics, Valencia, Spain ;
[3]Harvard School of Medicine- Harvard University-, Department of Obstetrics and Gynecology, Boston MA, U.S.A. ;
[4]Valencia University- and INCLIVA, Department of Obstetrics and Gynecology, Valencia, Spain

Study question: What is the Detection Rate (DR) and the clinical utility of Expanded Carrier Screening (ECS) application on the management of patients trying to conceive?

Summary answer: The high DR of At-Risk Couples (ARCs) and the remarkable proportion opting for IVF/Preimplantation Genetic Testing (PGT) treatment demonstrate ECS clinical utility in reproductive contexts.

What is known already: Around 1-2% of couples are at risk of conceiving a child with an autosomal recessive or X-linked disorder. Carrier screening (CS) identifies ARCs improving their reproductive autonomy. Previously, CS was employed to test single diseases (e.g., Cystic Fibrosis). Current genetic technologies allow simultaneous/parallel testing of multiple disorders. However, the previous low cost-effectiveness, suboptimal construction of test panels and inexperience with such genetic analysis across medical professionals are still undermining mainstream application of ECS. Here, a minimal ECS gene-panel was routinely applied on a large population of reproductive patients providing evidence of its DR, clinical utility and cascade testing opportunity

Study design, size, duration: A total of 2,013 individuals without family history of genetic disorders were enrolled at affiliated clinics in different Italian regions between March 2017 and December 2019. Of these, 1,172 individuals were from couples trying to conceive (902 through In Vitro Fertilization (IVF) and 270 by natural conception). Additionally, 181 individuals undergoing homologous IVF, 636 heterologous IVF and 24 natural conception were enrolled through *One-Member Screening Strategy*. Data were collected and elaborated anonymously following IRB approval.

Participants/materials, setting, methods: Monogenic disorders included in our panel (n=10) were selected based on ACMG-recommended criteria (prevalence, carrier rate, severity) and unequivocally associated with highly penetrant childhood conditions. Analytical methods employed include qPCR/Taqman assays for SNPs/indel variants, triplet-primed PCR and melting curve analysis for expansions and qPCR-based CNV analysis for exons/introns deletions. Couples were defined at-risk when both partners carried an autosomal recessive pathogenic variants on the same gene or when woman was a carrier of X-Linked disorder.

Main results and the role of chance: A total of 174 patients (8.6%) were identified as carriers of one of tested pathogenic variants: CFTR (n=59, 2.9%); SMN1 (n=46, 2.3%); DHCR7 (n=23, 1.1%); PMM2 (22, 1.1%); ACADM (n=15, 0.7%); HADHA (n=4, 0.2%); FMR1 (n=3, 0.4%); ARSA (n=1, 0.05%) and DMD (n=1, 0.1%). Remarkably, across the 586 couples tested, 13 (2.2%) were found to be at increased risk of having an affected child (ARCs). In particular, the identified ARCs were carriers of Cystic Fibrosis (n=5, 0.8%), Spinal Muscular Atrophy (SMA) (n=3, 0.5%), Fragile-X Syndrome (n=3, 0.5%), Smith-Lemli-Opitz Syndrome (n=1, 0.2%) and Duchenne/Becker Dystrophy (n=1, 0.2%). Notably, 3 of these ARCs were identified among couples trying to conceive by natural conception. Moreover, the identification of a patient carrying a pathogenic variant on the SMN1 gene led to cascade testing of his relatives, revealing another ARC for SMA in the family. At present, among ARCs that were followed up, all of them (N=10) pursued actions to reduce their reproductive risk by undergoing IVF with PGT for monogenic conditions (PGT-M). To date, 4/10 (40.0%) couples completed their PGT-M cycle with euploid/healthy embryos. Two of these couples have already achieved a healthy pregnancy after embryo transfer.

Limitations, reasons for caution: The main limitation of this study involves the lack of ethnic diversity in our cohort (mainly Caucasians). Also, the use of a limited panel of core gene-disease pairs can underestimate the rate of carriers and ARCs detectable in this cohort of individuals.

Wider implications of the findings: Extensive application of ECS detects a remarkable proportion of ARCs, improving couples' reproductive autonomy and providing opportunities for cascade genetic analysis. Continuous reduction in sequencing costs and improvement in variant interpretation and genotype-phenotype association are predicted to widen the diagnostic scope of ECS, leading to increasingly higher detection rates.

Trial registration number: not applicable

O-270 A novel long-range DNA sequencing approach improves the design of new protocols for preimplantation genetic testing of monogenic disease (PGT-M).

M. Leaver[1], N. Kubikova[1], X. Tao[2], C. Jalas[2], D. Wells[3]

[1]University of Oxford, Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom ;
[2]The Foundation for Embryonic Competence, New Jersey, New Jersey, U.S.A. ;
[3]Juno Genetics, Oxford, Oxford, United Kingdom

Study question: Can long-range next-generation sequencing assist in the work-up of PGT-M cases, with potential to increase patient access, improve accuracy and reduce costs?

Summary answer: Long-range DNA sequencing provides a powerful, low-cost method that reveals the informative SNPs closest to gene mutations and eliminates the need for additional family samples.

What is known already: To avoid misdiagnoses caused by allele dropout (failure to amplify one of the two alleles in an heterozygous cell), PGT-M strategies usually involve parallel analysis of several diagnostically relevant sites (e.g. mutations+linked polymorphisms). Polymorphisms need to be as close as possible to mutation sites because of the possibility of recombination. To determine which polymorphic alleles are associated with the disease, DNA from additional family members is usually analysed. However, relatives are not always available, as patients may carry *de novo*mutations, relatives may be untested or deceased, or patients may not wish to disclose their PGT-M treatment to others.

Study design, size, duration: A novel work-up method for PGT-M was evaluated. DNA was obtained from 13 couples undergoing PGT for different monogenic disorders. For each mutation, two primer sets (A and B) were designed, allowing amplification of the mutation plus an additional ~10kb upstream (A) or downstream (B). Amplicons were sequenced as single contiguous reads using the MinION (Oxford Nanopore). This identified informative single nucleotide polymorphisms (SNPs) and revealed which alleles exist on the same chromosome as mutations.

Participants/materials, setting, methods: 10kb regions flanking (and encompassing) mutation sites were sequenced from the 26 patients. Traditionally, candidate linked polymorphisms are identified from databases, but many turn out to be uninformative. Alternatively, parental samples can be analysed by microarray, simultaneously assessing many SNPs, but only evaluating a fraction of the variations in the genome. The SNPs found to be informative are sometimes relatively distant from the affected gene, increasing the chances of recombination between the SNP and mutation.

Main results and the role of chance: All 18 mutations in the 13 couples were successfully detected using long-range sequencing. Additionally, between 2 and 83 (average 18) informative SNPs were found in the 10kb flanking regions. The average distance from mutation sites to the nearest informative SNPs was 2529bp.This compares to an average distance of 32,179bp when microarrays were used to identify suitable SNPs. The extremely close proximity of

polymorphisms identified by long-range sequencing means that diagnostic challenges due to separation of SNPs from the disease-causing mutation by meiotic recombination can be virtually ruled out. In 28% of patients the closest informative polymorphism had a minor allele frequency <0.1. Such SNPs are rarely heterozygous and are therefore unlikely to be included in SNP-microarrays and are also unlikely to be chosen from databases as a candidate marker for PGT-M work-up.Additionally, novel informative intragenic SNPs, not present in any database, were identified in three couples. Because mutations and SNP alleles were contained within the same sequencing 'read', phasing was successfully accomplished in all cases, without any need for samples from additional family members. This will be of great value for couples who have no relatives suitable for phasing of polymorphisms (approximately one quarter of all PGT-M couples).
Limitations, reasons for caution: Unlike generic PGT-M methods (e.g. haplarithmisis/karyomapping), this strategy requires a customised protocol for each couple. The use of direct mutation testing combined with analysis of the closest possible SNPs flanking the mutation site is an approach that is unsurpassed in accuracy, but requires primer design and therefore slightly more work-up.
Wider implications of the findings: By providing a simple, inexpensive, rapid method of identifying the closest informative polymorphisms to parental mutations, long-range sequencing potentially improves PGT-M accuracy, reduces costs of customised protocols and accelerates test development. Furthermore, this approach removes the need to obtain DNA from any family members other than the couple undergoing PGT.
Trial registration number: N/A

O-271 KDM1A mediates transgenerational metabolic disturbances in a sex-specific manner and is linked to diet-induced altered sperm chromatin signatures

A.S. Pepin[1], C. Lafleur[2], V. Dumeaux[3], D. Sloboda[4], S. Kimmins[2]

[1]McGill University, Department of Pharmacology and Therapeutics, Montreal, Canada ;
[2]McGill University, Department of Animal Science, Montreal, Canada ;
[3]Concordia University, PERFORM Centre, Montreal, Canada ;
[4]McMaster University, Department of Biochemistry and Biomedical Sciences, Hamilton, Canada

Study question: Our objectives were to investigate whether epigenome-environment interactions can lead to enhanced metabolic phenotypes transgenerationally, and whether transmission of phenotypes are linked to sperm chromatin signatures.
Summary answer: Combining a genetic model of epigenetic inheritance with a diet-induced obesity model led to sex-specific transgenerational metabolic disturbances and aberrant sperm chromatin profiles.
What is known already: Obesity occurs in 650 million people worldwide (World Health Organization, 2016). Known factors contributing to obesity risks include genetics, lifestyle and maternal factors. Epidemiological studies and animal model data indicate that paternal diet also affects offspring risks to develop adult-onset metabolic disorders. However, the mechanisms underlying this non-genetic inheritance of complex metabolic disease remain elusive. Sperm histone methylation has been implicated in transgenerational epigenetic inheritance and offspring health (Siklenka et al. 2015). This was previously demonstrated using a transgenic mouse model that overexpresses the histone demethylase KDM1A specifically in the germline, giving rise to males with an abnormal sperm epigenome.
Study design, size, duration: KDM1A transgenic males with a pre-existing compromised sperm epigenome and C57BL6NCrl wildtype sires, were exposed to either a low- or high-fat diet (10% or 60% kcal fat, respectively) for two spermatogenic cycles (10-12 weeks). The next generations were generated by mating males with chow-fed C57BL6NCrl females. All animals were subjected to a series of metabolic tests at 4 months of age (F0: n=15-25 animals per group, F1: n=28-49, F2: n=8-21) and sacrificed at 5 months.
Participants/materials, setting, methods: Metabolic assessment was performed using glucose and insulin tolerance tests and baseline blood glucose levels. At necropsy, mesenteric adipose depots were weighted to assess adiposity. Liver RNA was extracted (n=4-6 per sex per group per generation) for differential gene expression. Sperm of F0 sires (n=5 per group) was subjected to chromatin immunoprecipitation sequencing targeting histone H3 methylation and analyzed to identify diet-modulated changes in enrichment which could be implicated in offspring phenotypes.
Main results and the role of chance: Sires (F0) fed a high-fat diet became obese, glucose intolerant and insulin insensitive, with increased adiposity, irrespective of their genotype. Intergenerational effects of paternal diet were observed in male offspring only, while metabolic functions in female descendants were not impacted by paternal high-fat diet. Males sired by obese transgenics had enhanced metabolic phenotypes compared to wildtype obese descendants. Interestingly, transgenerational effects of high-fat diet were only observed in transgenic descendants, suggesting that paternal exposure to a multitude of environmental stressors may exacerbate descendants' risk for obesity and metabolic syndrome. Sex-specific effects were further observed in the F2 generation, suggesting males may be more susceptible to paternal and grand-paternal diets. Sperm chromatin profiling revealed diet-induced alterations in enrichment. Differentially enriched regions occurred at genes with functions corresponding to the observed offspring phenotypes, including genes involved in placenta development, as well as glucose and lipid metabolism.
Limitations, reasons for caution: Further studies are required to better understand the molecular mechanisms underlying the links between diet-induced obesity, an aberrant sperm epigenome and offspring metabolic functions.
Wider implications of the findings: This is the first report linking high-fat feeding and alterations in the sperm epigenome at the level of a histone modification. These findings shed light on the potential contribution of chromatin in sperm in paternal transmission of complex diseases.
Trial registration number: not applicable

O-272 Germline Characterization of Genes Associated with Spermatogenesis and Embryonic Developmental Competence in Azoospermic Men

S. Cheung[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Can DNA sequencing of spermatozoa from azoospermic men identify germline mutations related to the etiology of their infertility and ability to support a pregnancy?
Summary answer: Men with secretory azoospermia have mutated key genes that impair sperm production and affect the ability of their spermatozoa to support embryonic development.
What is known already: Azoospermia accounts for about 15% of male factor infertility cases. Although it can be caused by pre-testicular factors, the most recognized forms are testicular and post-testicular. Post-testicular azoospermia, the most severe form, is characterized by scattered functional germinal epithelia that strive to support the meiotic process during gamete development. To shed light on the etiology of this condition, genetic studies have been performed, exclusively on peripheral blood. We performed a genetic assessment of the spermatozoa to preferentially detect germline mutations that may be passed onto offspring.
Study design, size, duration: During the last 18 months, we performed DNAseq on epididymal and testicular spermatozoa from men with acquired azoospermia (OA) (n=17) and nonobstructive azoospermia (NOA) (n=10), respectively, as well as on the ejaculated gametes from 3 fertile donor controls. Gene mutations related to azoospermia origin were categorized and compared. Gene mutation profiles of the OA and NOA men were then assessed in relation to their ability to generate a pregnancy (fertile) or not (infertile).
Participants/materials, setting, methods: DNA was extracted and amplified from at least 500 spermatozoa (DNA concentration, 705±562 ng/ul; quality, 1.7±0.1 nm). Following NGS, gene mutations, duplications, and deletions were detected using the CLC Genomic Server 9.0. Genes were considered duplicated or deleted when the read depth was >1.5 or <0.5 times the median read depth in the control. Common gene mutations from the OA and NOA cohorts were assessed according to the couples' clinical outcome.
Main results and the role of chance: Of the 27 couples (paternal age, 41.3±5yrs) included in this study, 17 OA men underwent surgical sperm retrieval, with an average concentration of 1.3±3x10^6/ml and 7±14% motility. Ten NOA men yielded spermatozoa with a concentration of 0.03±0.2x10^6/ml and 0.5±1% motility. NGS assessment did not show a significant difference in overall sperm aneuploidy between the two groups (OA, 1.7%; NOA, 1.9%).

In the OA group overall, only 3 housekeeping genes were mutated (ATP4A, SLC17A7, and OR1D4). In the NOA patients, however, 5 genes involved in RNA transcription (POLR2L), apoptosis (AP5M1), and basic spermiogenic function (AP1S2, AP1G2, and APOE) were deleted.

The OA patients were treated in 17 ICSI cycles (maternal age, 34.8±3yrs), resulting in a pregnancy and delivery rate of 47.1% (8/17). The fertile and infertile OA cohorts had only 1 mutated gene, ZNF749 and PRB1, respectively. Both were unrelated to spermatogenesis or embryo developmental competence.

When NOA men were treated in 10 ICSI cycles (maternal age, 38.2±2yrs), the pregnancy rate was 70% (7/10). While the fertile cohort displayed 1 mutated gene (MPIG6B) related to stem cell lineage differentiation, the infertile NOA cohort had deleted genes involved in spermatogenesis (n=6), apoptosis (n=4), acrosomal function (n=2), and early embryonic development (n=8).

Limitations, reasons for caution: This is a novel study with a limited number of observations, and it cannot demonstrate genetic association with obstructive azoospermia independently of reproductive performance. In addition, although female partner age was controlled for, confounding factors cannot be excluded with certainty.

Wider implications of the findings: This novel DNAseq study aims to identify germline mutations. The reproductive performance of OA men was not associated with any genetic mutations. In the NOA cohort, fertile men had only one deleted gene involved in sperm production, while infertile men had several deleted genes involved in spermatogenesis and embryonic development.

Trial registration number: not applicable

O-273 Knowledge, attitudes and preferences regarding expanded carrier screening among reproductive-aged men and women in Belgium

E. Van Steijvoort[1], I. Geysen[1], S. Van Epperzeel[1], H. Devolder[1], H. Peeters[2], K. Peeraer[3], G. Matthijs[2], P. Borry[1]

[1]KU Leuven, Department of Public Health and Primary Care, Leuven, Belgium ;
[2]KU Leuven, Department of Human Genetics, Leuven, Belgium ;
[3]KU Leuven, Department of Development and Regeneration, Leuven, Belgium

Study question: What is the knowledge, attitudes and preferences regarding expanded carrier screening among reproductive-aged men and women in Belgium?

Summary answer: Health care providers and policy makers should take the current knowledge level, attitudes and preferences of potential users in account when implementing ECS.

What is known already: Through carrier screening couples at-risk of conceiving a child with an autosomal recessive or X-linked condition can be identified prior to conception, allowing prospective parents to make informed reproductive decisions when planning for a family. In the last decade, expanded carrier screening has become available to (prospective) parents. Following recommendations made by the Superior Health Council (SHC) of Belgium, a Belgian Genetic Carrier Screening test (BeGECS) was launched in October 2019. However, little is known about the interest, knowledge level, attitudes and preferences of potentials users. More insights are need to ensure responsible implementation of the BeGECS offer.

Study design, size, duration: A cross-sectional survey was conducted using convenience sampling. Individuals of reproductive age visiting their public pharmacist were invited to answer a self-administered questionnaire assessing knowledge, attitudes and preferences regarding expanded carrier screening (ECS). Prior to filling in the questionnaire, participants were asked to read an information letter explaining some key concepts. Based on our sample size calculation we aimed to collect 385 completed questionnaires. Data collection was carried out between September 2019 and December 2019.

Participants/materials, setting, methods: The study population consisted of reproductive aged (18-49 years) men and women. Participants were recruited through five public pharmacies in Flanders (Belgium). Potential participants were approached about the study by researchers present in the pharmacies and were asked to fill in the anonymous questionnaire on the spot after reading an information sheet explaining some key concepts. The questionnaire contained questions on socio-demographic characteristics, risk perception, intention to have ECS, knowledge, attitudes, preferences, etc.

Main results and the role of chance: Our sample (n=387) consists of 265 females (68.5%) and 122 males (31.5%). Most of the participants were below 34 years old (72.9%), didn't have children (68.6%) and were currently in a relationship (69.1%). Of those who were in a relationship, 52.1% had a future child wish. More than half of the participants (53.6%) estimated their chance of being a carrier for a recessive disorder (very) low. Likewise 64.8% of participants estimated their chance of conceiving a child with a recessive disorder (very) low. Offering ECS to couples with a child wish was found acceptable by 86% of participants. However, fewer participants would consider ECS for themselves in the future (61%). Only 19 (4.9%) participants answered all knowledge questions correctly. Half of the participants (50.9%) preferred the disclosure of individual results, while 35.2% preferred couple-based results. Most participants indicated that ECS should be offered through the gynecologist (81.1%), followed by the GP (71.5%) and the Center for Human Genetics (64.8%). About 68.9% of participants were willing to pay out-of-pocket for an ECS test. If participants were willing to pay themselves, they indicated that the test should have a maximum cost of 150 euros (45.3%) or between 150 – 300 euros (40%).

Limitations, reasons for caution: Our study employed convenience sampling to recruit participants, therefore our reported results should be interpreted with caution. Another limitation of our study is that we didn't offer an actual ECS-test to participants so some results are only hypothetical. Actual participation might differ from the intention to do a behavior.

Wider implications of the findings: Our study reports on the views of the potential users of ECS. These results can guide health care providers and policy makers when implementing ECS in the future to ensure that couples makes informed reproductive decisions based on accurate knowledge and consistent with the values of the couple.

Trial registration number: Not applicable

O-274 Single nucleotide polymorphisms (SNPs) in FSHR/FSHβ genes do not modify ovarian response to stimulation with rFSH. A prospective multicentre study in Europe and Asia

N.P. Polyzos[1], C. Blockeel[2], C. Spits[3], B. Alvaro Mercadal[1], S. Garcia[4], P. Ma[5], L. Le[5], M. Ho[5], G. Duqué[3], J. Mertens[3], D. Stoop[6], P. Drakopoulos[2], H. Tournaye[2], F. Martinez[1], N. Vuong[7]

[1]Dexeus University Hospital, Reproductive Medicine Department, Barcelona, Spain ;
[2]Universitair Ziekenhuis Brussel-Vrije Universiteit Brussel, Centre for Reproductive Medicine, Brussels, Belgium ;
[3]Vrije Universiteit Brussel, Research Group Reproduction and Genetics, Brussels, Belgium ;
[4]Dexeus University Hospital, Statistics Department, Barcelona, Spain ;
[5]My Duc Hospital, IvfMD, Ho Chi Minh City, Vietnam ;
[6]Ghent University Hospital, Centre for Reproductive Medicine, Ghent, Belgium ;
[7]University of Medicine and Pharmacy, Medical Statistics Department, Ho Chi Minh City, Vietnam

Study question: Does the presence of SNPs in FSHR/FSHβ influence oocyte yield and Follicular output rate (FORT) in predicted normal responders treated with rFSH?

Summary answer: The presence of SNPs in FSHR/FSHβ (rs6165, rs6166, rs1394205, rs10835638) does not influence ovarian response in predicted normal-responders treated with a fixed-dose of 150IU rFSH

What is known already: Ovarian reserve markers have been a breakthrough in response prediction following ovarian stimulation. However, a significant percentage of patients show a disproportionate lower ovarian response, as compared with their actual ovarian reserve. Studies on pharmacogenetics demonstrated a relationship between FSHR/FSHβ genotyping and drug response, suggesting a potential effect of individual genetic variability on ovarian stimulation. However, evidence from these studies is inconsistent, due to inclusion patients with variable ovarian reserve, use of different starting gonadotropin doses and allowance for dose adjustments during treatment. This highlights the necessity of a well-controlled prospective study, in homogenous population treated with an identical protocol.

Study design, size, duration: We conducted a multicenter multinational prospective study, including 368 patients from Vietnam, Belgium and Spain (168 from Europe and 200 from Vietnam), from November 2016 till June 2019. All patients underwent ovarian stimulation followed by oocyte retrieval in an antagonist protocol with fixed daily dose of 150IU of rFSH until triggering. Blood

sampling and DNA extraction was performed prior to oocyte retrieval, followed by genotyping of 4 SNPs from FSHR/FSHβ (rs6165, rs6166, rs1394205, rs10835638).

Participants/materials, setting, methods: Eligible were predicted normal responder women <38 years old undergoing their 1st/2nd ovarian stimulation cycle. Laboratory staff and clinicians were blinded to the clinical results and genotyping respectively. The number of oocytes and the FORT (number of follicles on trigger day/AFCx100) were compared between different FSHR/FSHβ genotypes. Results were analysed with x^2 or oneway ANOVA and were corrected for multiple comparisons as appropriate.

Main results and the role of chance: The prevalence of homozygous SNPs in the FSHR was: rs6166(c.2039 A>G) 15.8%, rs6165(c.919 A>G) 34.8% and rs1394205(c.-29G>A) 14.1% with significant differences between European and Asian women. FSHβ rs10835638(c.-211 G>T) was very rare: 0.5%.

Comparison between different SNPs and genotypes, wild-type (WT), heterozygous(HT) or homozygous(HZ), did not reveal significant differences in terms of ovarian response.

The number of oocytes was comparable between the 3 genotypes (WT vs.HT vs.HZ) for all 3 variants in FSHR :

1. a. rs6166 (mean(SD)): 14.81(7.07) vs. 13.49(7.30) vs. 13.69(5.82), p=0.066
2. b. rs6165: 14.39(7.08), 13.59(7.54), 14.25(6.30), p=0.095
3. c. rs1394205: 15.06(8.10), 13.24(5.89), 13.61 (6.95), (p=0.088)

Similarly, no differences were observed on the FORT for rs6166 (p=0.814), rs6165 (p=0.974) and rs1394205 (p=0.081). Linear regression analysis to predict the number of oocytes in relation to genetic variants (rs6166, rs1394205, rs6165) adjusted for age, AMH, continent and duration of stimulation, did not show differences between WT, HT, HZ for rs6166 and rs6165. Although rs1394205 HZ had statistically significant lower oocyte yield compared with WT, this was of minimal clinical significance, 0.84 95%CI [0.71;0.98] oocytes less.

Finally, the proportion of women with optimal response (>10 oocytes) was also comparable between the 3 groups (WT vs. HT vs. HZ) for rs6165, rs6166 and rs1394205

Limitations, reasons for caution: The study was performed in relatively young women with normal ovarian reserve to eliminate biases related to age-related fertility decline; thus, caution is needed when extrapolating results to other populations. In addition, no analysis was performed for FSHβ rs10835638 due to the very low prevalence of homozygotes (n=2).

Wider implications of the findings: Based on our results, genotyping SNPs rs6165, rs 6166, rs1394205, rs10835638 prior to initiating an ovarian stimulation with rFSH in predicted normal responders should not be recommended since their presence does not modify ovarian response in these women. Future research may focus on other genes related to folliculogenesis or steroidogenesis

Trial registration number: NCT03007043

INVITED SESSION

SESSION 65: BIOMARKERS OF FAILED PREGNANCY

08 July 2020 Parallel 1 12:00 - 13:00

O-275 Biomarkers of failed pregnancy (miscarriage ectopic). The clinician's perspective

A. Daponte[1], O. Koukoura[2]

[1]*University of Thessaly- School of Health Sciences- Faculty of Medicine, Obstetrics and Gynaecology, Larissa, Greece*

[2]*University Hospital of Larissa, Obstetrics and Gynaecology, Larissa, Greece*

Abstract text

Ectopic pregnancy (EP) occurs when a blastocyst abnormally implants outside the endometrium The fallopian tube is the commonest location in more than 95% of cases The prevalence of EP has doubled since 1960 and accounts for about 2% of the pregnancies in the first trimester. In recent years, its incidence has increased due to the increase in incidence of pelvic inflammatory diseases, use of fertility drugs, and pelvic surgery. Transvaginal ultrasound and serial measurement of serum beta-hCG levels are the most common diagnostic methods for EP. Despite the use of transvaginal ultrasound and measurement of beta-hCG levels, about 40% to 50% of the initial cases of the disease are not diagnosed. Therefore, the need for new diagnostic tools, preferably serum biomarkers, is essential.

Several diagnostic biomarkers for EP have been proposed over the past decades. Progesterone along with beta-hCG are the first and widely used biomarkers. Progesterone however, although represents a good indicator of early pregnancy viability, is a poor predictor of pregnancy location. To produce the optimal biomarker for early EP detection, several obstacles must be overcome. Firstly the behaviour of an EP and the substances produced during the first few days following implantation, might vary considerably in cases where the pregnancy is a result of assisted reproduction technology, the site of implantation and whether the EP is intact ruptured or aborted. Investigators studied several biomarkers produced by the fallopian tube, the early embryo, the endometrium, the ovary, or peritoneal inflammatory factors. So far none of these substances have been widely used in clinical practice.

Cancer antigen 125 has been reported as a marker for pregnancies "likely to continue". Inhibin A, activin A, and high sensitivity C-reactive protein (hsCRP) were not significantly different in women with successful and failed expectant management. On the other hand, inhibin A may be useful for predicting spontaneous resolution of pregnancy of unknown location (PUL) but is not as good as progesterone. Recent attention has focused on metalloprotease 12 (ADAM 12) and fibronectin as potentially promising candidate markers in PUL pregnancies. Serum Interleukin-15 (IL-15) and anti-C1q antibodies although showed promising results in differentiating between an abortion and EP, they had no further clinical application. Similar results presented when placental growth factor (PlGF) and the soluble Flt-1 (sFlt-1) receptor of vascular endothelial growth where investigated.

Several studies outline the utility of creatine phosphokinase (CPK), which is an intracellular enzyme found in the fallopian tube, as a marker for early diagnosis of EP. The theory behind the idea is that since CPK is an intracellular enzyme, lysis of trophoblast cells leads to an increase in plasma CPK levels. Therefore, the level of this enzyme can be used for evaluation of tubal ectopic pregnancy because increased CPK can be associated with trophoblastic invasion and trophoblast mass. However different studies produced conflicting results.

Metabolomics and epigenomics represent two rather new fields of research in medicine. Metabolomic markers have been produced promising results in recent studies. Epigenetic studies are mainly focused on detecting micro-RNAs that regulate expression of EP-associated genes such as VEGFA, EGFR, ESR1 and immune response-related genes. Most of these studies have a limited number of subjects, therefore, their results need to be confirmed by future studies.

The ideal diagnostic tool for an EP would be a single serum marker to replace ultrasound and serial biochemistry. This implies earlies management and the opportunity for conservative management in most cases. To date this ideal serum biomarker is under investigation.

Trial registration number: -

Study funding: -

Funding source: -

O-276 Developing biomarkers for non-viable pregnancies: the laboratory expert perspective

A. Horne[1]

[1]*University of Edinburgh, MRC Centre for Reproductive Health, Edinburgh, United Kingdom*

Abstract text

The development of biomarkers for prompt diagnosis, and safe timely treatment, of non-viable pregnancies is essential. A blood-based biomarker that accurately identifies an ectopic pregnancy could be used to offer early diagnostic certainty in cases where ultrasound cannot determine the location of the embryo ('a pregnancy of unknown location'). Here, I review the literature on the development of molecularly targeted diagnostics for ectopic pregnancy, miscarriage and viable pregnancies. Molecules examined so far can be broadly grouped into biological themes of relevance to reproduction: (i) Fallopian tube (dys)function,

(ii) embryo/trophoblast growth, (iii) corpus luteum function, (iv) inflammation, (v) uterine function and (vi) angiogenesis. While a sensitive and specific biomarker for ectopic pregnancy has yet to be identified, it is possible that improvements in platform technologies or a multi-modal biomarker approach may yield an accurate diagnostic biomarker test. Furthermore, with the advent of better imaging technology, the need for a blood-based biomarker test may be superseded by improvements in ultrasound or magnetic resonance imaging technology.

INVITED SESSION

SESSION 66: SYNTHETIC EMBRYOLOGY: MYTH OR REALITY?

08 July 2020 Parallel 2 12:00 - 13:00

O-277 Modelling blastocyst formation and in utero implantation solely from stem cells

N. Rivron[1]

[1]*IMBA- Austrian academy of science, Laboratory for synthetic development, Vienna, Austria*

Abstract text

The pre-implantation conceptus (blastocyst) forms all embryonic and extra-embryonic tissues. It consists of a spherical thin-walled layer, the trophectoderm, that surrounds a fluid-filled cavity sheltering the embryonic and primitive endoderm cells. From mouse blastocysts, both trophoblast and embryonic stem cell lines can be derived, which are in vitro analogues of the trophectoderm and embryonic compartments, respectively. Our lab showed that trophoblast and embryonic stem cells self-organize in vitro to form structures that morphologically and transcriptionally resemble blastocysts (blastoids). Blastoids form primitive endoderm-like cells, the second extra-embryonic lineage, and implant upon in utero transfer. Like blastocysts, blastoids form via inductive signals originating from the inner embryonic cells and driving outer trophectoderm development. However, the nature and function of these molecular signals are largely unexplored. Genetically and physically uncoupling the embryonic and trophectoderm compartments, along with single cell transcriptomics, revealed an extensive list of inductive signals. We show that the embryonic cells maintain trophoblast proliferation and self-renewal, while fine-tuning trophoblast epithelial morphogenesis. Altogether, these embryonic inductions are paramount to form a trophectoderm state that robustly implants and triggers a genuine decidualization in utero. Thus, at this stage, the nascent embryo fuels the development and implantation of the future placenta. Altogether, the blastoid is a powerful embryo model that can be reproducibly generated in large numbers, finely tuned, contains all the cell types to form the conceptus, and implants in utero.

References:

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Frias-Aldeguer J. et al. Embryonic signals perpetuate polar-like trophoblast stem cells and pattern the blastocyst axis. BioRxiv 510362; doi: https://doi.org/10.1101/510362

Vrij E. et al. Chemically-defined induction of a primitive endoderm and epiblast-like niche supports post-implantation progression from blastoids. BioRxiv 510396; doi: https://doi.org/10.1101/510396.

O-278 Stem cells in reproductive biology: myth or reality?

G P. Schatten[1]

[1]*University of Pittsburgh, Department of OB/GYN & Reproductive Sciences & Cell Biology & of Bioengineering; UP Cancer Institute, U.S.A*

INVITED SESSION

SESSION 67: COVID-19 - PSYCHOSOCIAL IMPACT OF DELAYED TREATMENT

08 July 2020 Parallel 3 12:00 - 13:00

O-279 Impact of COVID-19 pandemic on the psychological status of infertile patients who had in vitro fertilization treatment interrupted or postponed

S. Ferrero[1], **C. Scala**[2], **M. Altieri**[1], **F. Barra**[1]

[1]*IRCCS Ospedale Policlinico San Martino- University of Genoa, Academic Unit of Obstetrics and Gynecology, Genoa, Italy*

[2]*Gaslini Hospital, Unit of Obstetrics, Genoa, Italy*

Study question: To understand the changes in the psychological status of infertile patients who had in vitro fertilization (IVF) treatment interrupted or postponed because of the COVID-19 pandemic.

Summary answer: Infertile women who had IVF treatment postponed because of COVID-19 pandemic have psychological symptoms. IVF centers must strengthen psychological counseling to improve patients' mental health.

What is known already: Infertility is a severely distressing experience for many couples. Depression and anxiety are psychological disorders associated with infertility, and they may worsen during infertility treatments.

Study design, size, duration: This study was conducted in April 2020. A survey's link was e-mailed to the partecipants. A reminder e-mail was sent every week for one month.

Participants/materials, setting, methods: This study included all the patients attending our institution who had IVF treatment interrupted or delayed because of the COVID-19 pandemic. The severity of anxiety and depression was assessed using the Generalized Anxiety Disorder-7 (GAD-7) and the Patient Health Questionnaire-9 (PHQ-9). Subjects with pre-existing psychiatric disorders were excluded.

Main results and the role of chance: 503 patients (294 women and 209 men) completed the questionnaire (response rate: 80.9%; n = 622). The mean (± SD) age of the women included in the study was 37.3 (± 4.6) years; the mean age of men was 38.4 (±6.2). The median number of previous failed IVF cycles was 2 (range, 0-8). The prevalence of anxiety or depression, or both was 37.0% (186 of 503). The prevalence of anxiety or depression or both was significantly higher in women (41.5%; 95% C.I. 35.8%-47.4%) than in men (30.6%; 95% C.I., 24.4%-37.4%; p = 0.012). The median total score on GAD-7 was 11 (interquartile range, IQR: 9-14). The median total score on PHQ-9 was 10 (IQR: 9-14). The occurrence of anxiety and/or depression was significantly associated with a diagnosis of poor ovarian reserve (odds ratio, OR=2.13), diagnosis of endometriosis (OR=1.54), diagnosis of uterine fibroids (OR=1.34) and time spent on COVID-19 related news per day (> 1 hour per day, OR=1.47). Notably, 187 patients (37.2%; 32.9%-41.6%) declared that they would like to undergo the IVF treatment despite the COVID-19 pandemic. 384 patients (76.3%; 72.4%-80.0%) declared that they would be reassured if they knew when they could restart the IVF treatment.

Limitations, reasons for caution: A longitudinal follow-up would be helpful in tracking the changes in anxiety and depression levels at different stages of the epidemic.

Wider implications of the findings: This study reveals that the COVID-19 epidemic caused a sharp increase in the prevalence of anxiety and depression among infertile patients undergoing IVF. IVF center must strengthen the psychological counseling for these patients to improve their sleep quality and mental health.

O-280 Patient experiences of fertility clinic closure during the COVID-19 pandemic: Appraisals, coping and emotions

J. Boivin[1], **C. Harrison**[1], **R. Mathur**[2], **G. Burns**[3], **A. Pericleous-Smith**[4], **S. Gameiro**[1]

[1]*Cardiff University, School of Psychology, Cardiff, United Kingdom*

[2]*Manchester University NHS Foundation Trust and Manchester Academic Health Sciences Centr, OB-Gyn, Manchester, United Kingdom*

[3]*Fertility Network UK, Director, London, United Kingdom*

[4]*British Infertility Counselling Association, Chair, York, United Kingdom*

Study question: What are appraisals, coping strategies and emotional reactions to COVID-19 fertility clinic closure?

Summary answer: Clinic closure was appraised as stressful due to uncertainty and threat to attainability of parenthood goal but patients were able to cope using accommodative strategies.

What is known already: According to stress and coping theory, imbalance between threat and coping resources leads to stress reactions. People facing disasters generally experience more stress than usual, but remarkably most are able to cope and recover, with some eventually seeing benefits from the situation. Research to date on experiences of COVID-19 in the general population indicates more anxiety and depression among respondents than historical norms, worry about becoming mentally unwell due to uncertainty and loss of control but nevertheless ability to cope. Reports of reactions to COVID-19 in infertility patients shows these are moderately to extremely upset about COVID-19 treatment cancellation.

Study design, size, duration: Cross-sectional design. Mixed-methods, English, anonymous, online survey posted from April 09 to April 21 to social media. Eligibility criteria was being affected by COVID-19 fertility clinic closures, 18 years of age or older and able to complete survey in English. In total 946 people clicked on the survey link, 76 did not consent, 420 started the survey but did not complete it, and 450 completed the survey (48% completion, 446 women, 4 men).

Participants/materials, setting, methods: On average participants were 33.6 years (SD=4.4) and had been trying to achieve pregnancy for 3.5 years (SD=2.22). The survey comprised quantitative questions about intensity of threat, emotions, and ability to cope with clinic closure. Open-text questions covered understanding of COVID-19, fears, concerns and perceived benefits of clinic closure, and desired information about closure and future re-opening. Inferential statistics were used on quantitative data and thematic qualitative analysis (inductive coding) performed on open text data.

Main results and the role of chance: Overall 82.2% (n=367) had tests/treatments postponed. Patients appraised fertility clinic closure as significantly more negative than positive [$t(447)=45.2$, $p < .001$], and to be very or extremely uncontrollable [$t(445)=38.99$, $p < .001$] and stressful [$t(445)=27.44$, $p < .001$]. Most reported below average ability to cope with the closure (11.9% not at all able) [$t(445)=5.57$, $p < .001$]. Question by question textual analysis revealed 33 broad themes, grouped into four meta-themes across questions. First, COVID-19 effects were unknown and clinic closure perceived as precautionary or as unfair relative to advice about getting pregnant given to the public. Second, closure was appraised as a threat to attainability of the parenthood goal largely based on uncertainty of effects of delay and intensification of pre-existing hardships of fertility problems. Third, threat emotions and uncertainty taxed personal coping resources but most managed the situation using diverse strategies (e.g., thought-management, getting mentally and physically fit for next treatments, strengthening their social network, and keeping up-to-date with clinics. Finally, almost all participants reported significant stress, worry and frustration at the situation with a minority reporting intense feelings of hopelessness. More information about effects of delay and eventual wait-list prioritisation at re-opening was desired.

Limitations, reasons for caution: The survey captured reactions at a specific point in time (during lockdown before clinics announced re-opening) but results offered future directions for handling of information in COVID-19 conditions. Participants were self-selected, 25% non-UK countries and only 4 men all of which may affect generalisability.

Wider implications of the findings: Fertility stakeholders (e.g., clinics, patient groups, regulators, professional societies) need to work together proactively to address the continuing impact of COVID-19 on patients. Transparent processes for COVID-19 and sign-posting to information and coping support resources will bolster patients existing coping resources and support staff deliver the new normal for clinics.

O-317 A study to explore participants experiences of fertility services during the Covid-19 lockdown; a biphasic mixed methods study

B. Karavadra[1], **A. Balen**[2], **E. Morris**[1]

[1]*Norfolk & Norwich University Hospital, Obstetrics & Gynaecology, Norwich, United Kingdom*

[2]*Leeds Teaching Hospital, Obstetrics and Gynaecology, Leeds, United Kingdom*

Study question: What is the impact of Covid-19 on individuals using fertility services in the United Kingdom?

Summary answer: The implications of COVID-19 on individuals has been drastic and include uncertainty, delay in care and anxiety about pregnancy outcomes during the pandemic.

What is known already: COVID-19 has been declared a pandemic during which fertility services in the United Kingdom were temporarily suspended. Recently, services have started the process of resumption, but there remains significant uncertainty in the way fertility care will be delivered in the United Kingdom.

Study design, size, duration: The study involved two phases; an online questionnaire designed to obtain an overview of participants perceptions of Covid-19, impact on fertility care and perceived barriers to care from 422 participants. In phase two, pertinent themes from the questionnaire findings were explored further in greater depth and detail in 15 individual semi-structured interviews through purposive sampling in May 2020. The study was designed with patient and public engagement, as well as a clinicians and academics.

Participants/materials, setting, methods: The study was advertised via online platforms throughout the UK. Eligible participants included anyone who has experience of fertility services within the past one year. The 'open-ended' questionnaire results and transcribed semi-structured interview findings were analysed through thematic analysis (familiarisation with the data, creating initial codes, generating and reviewing subsequent themes, as well as refining themes in the context of the research question). Percentages were also used to compliment the qualitative findings.

Main results and the role of chance: 718 participants replied to the questionnaire with the majority identifying as White British (82%) and 5% as male. Over 60% had used fertility services within the last one year. 92% of individuals explained that COVID-19 had a 'negative impact' on their fertility treatment, namely 'delay in care'. 62% of participants discussed concerns about the 'uncertainty' they felt about fertility services; these included the 'unknown impact of COVID-19 on pregnancy outcomes', the 'unknown impact on general gynaecology services' and the 'unknown impact of COVID-19' on 'fertility success'. The influence of the media and its focus on COVID-19 at present was a perceived barrier to care as participants felt fertility care was 'less prioritised by the government'.

Through semi-structured interviews with fifteen participants, we learned about the concerns they had about the extension of the 10-year storage limit, particularly in relation to embryos. Interestingly, participants from a Black and Minority Ethnic (BAME) background discussed the 'cultural pressures' they faced during COVID-19 and the implications on their wider social circumstances. Participants were mindful about the 'pressures on the service' when re-opening, and therefore 'advancing maternal age', 'socio-economic background' and 'previous unsuccessful fertility treatment' were the main factors individuals considered important when 'prioritising' fertility care.

Limitations, reasons for caution: The questionnaire findings are representative mainly from individuals in the UK and from those identifying mainly as White British; subsequently, cultural influences that may affect participants views may not be representative. Those individuals without online connectivity may have had valuable insight but we were not able to capture this.

Wider implications of the findings: This study has truly enabled participants voices to be heard. The findings from this study can be used by fertility service providers to appreciate the patient perspective when considering the re-opening of fertility services nationally and internationally and be mindful of patient concerns.

O-318 Covid-19 does not stop fertility preservation: The Italian situation during the pandemic emergency.

A. Anastasi[1,8], **L. Sosa Fernandez**[2,8], **D. Cimadomo**[3,8], **F.G. Klinger**[4,8], **E. Licata**[5,8], **C. Scarica**[6,8], **L. De Santis**[7,8]

[1]*Hospital "del Delta", Physiopathology of Human Reproduction Center, Lagosanto- Ferrara., Italy*

[2]*Embryos Fertility Center, Reproductive Medicine, Battipaglia, Italy*

[3]*Clinica Valle Giulia - Genera Center, Reproductive Medicine, Rome, Italy*

[4]*University of Rome "Tor Vergata", Department of Biomedicine and Prevention-Section of Histology and Embryology, Rome, Italy*

[5]*Sandro Pertini Hospital, Physiopathology of Reproduction and Andrology Unit, Rome, Italy*

[6]Casa di Cura Villa Salaria in partnership with Institut Marques, Reproductive Medicine, Rome, Italy
[7]San Raffaele Scientific Institute- University Vita-Salute San Raffaele, Department of Obstetrics & Gynecology- IVF Unit, Milan, Italy
[8]On behalf of the Italian Society of Embryology, Reproduction and Research (SIERR)

Study question: How Coronavirus disease (Covid-19) influenced fertility preservation cycles in Italy? A SIERR (Italian Society of Embryology, Reproduction and Research) survey.
Summary answer: The survey carried out by SIERR shows how in Italy the pandemic influenced in a limited fashion fertility preservation treatments.
What is known already: At the beginning of March 2020 Italy was severely hit by SARS-CoV-2, whose rapid spreading has forced the government to impose the total lockdown of the country. Therefore, all ART centres in Italy decided to postpone their activities and reorganize their schedule to assure the safety for both patients and staff members. However, given the known reproductive risks of cancer therapies, there has been a special attention to fertility preservation. In fact, during the pandemic, most Italian ART centres guaranteed the execution of fertility preservation treatments. Currently, ART treatments are gradually restarting for any clinical indication.
Study design, size, duration: The aim of this longitudinal survey is to verify whether the pandemic has reduced the number of male and female fertility preservation procedures. The survey questionnaire consisting of fifteen questions (six multiple choice/nine brief answers), has been sent to all SIERR members on May 12th. On May 17th we no longer accepted responses for the first call. We will send a recall on May 22nd trying to collect a greater amount of data.
Participants/materials, setting, methods: In this highly stressful and demanding period, 46 SIERR members responded to the survey trough GoogleForm. Data obtained were analysed in order to receive one answer from each centre. Therefore, we collected answers from 42 centres. We asked our members how many fertility preservation cycles they have performed from 1st-January-2019 to 30th-April-2019 and how many in the same period of this year. The remaining questions regarded the management of these cycles during the pandemic.
Main results and the role of chance: Albeit a general reduction of the number of procedures in many medical fields, our data show that fertility preservation was not hampered by lockdown. Comparing the number of procedures during the same period in 2019 and 2020, results show a reduction of 19.6% in 2020. Interestingly, this reduction refers to the period previous Covid-19 emergency. In fact, from 1st-January-2019 to 30th-April-2019 Italian centres performed 626 fertility preservation cycles. Conversely, during the same period in 2020, 503 cycles were performed, of which 43% (74 female and 145 male preservation procedures) during the lockdown.

All patients have been screened with different methods: Triage (61%), RT-PCR (33%) or both screening methods (6%). Eleven centres performed female preservation cycles using open cryopreservation devices (91%) or closed system (9%). These samples have been stored in non-infected patients' tanks (81%) or in quarantine tanks (19%).

In 5 centres some members of the staff resulted positive to the virus; not surprisingly 3 out the 5 interested centres are located in Lombardia, the most affected region by SarsCoV-2 in Italy.
Limitations, reasons for caution: Unfortunately, due to the abstract deadline, the survey remained opened only for six days. ART Societies recommendation helped to ensure safety in the execution of fertility preservation cycles. However, in the short-term we recommend to keep the guard up in order to avoid the possibility of a return to phase1.
Wider implications of the findings: Many reasons may have caused the decrease in the number of cases comparing 2019 to 2020: some not urgent oncological surgeries have been probably postponed and consequently their fertility preservation procedures. Moreover, because during the lockdown it was strictly recommended to stay at home, many diagnoses might have been missed.

SELECTED ORAL COMMUNICATIONS
SESSION 68: GENETIC DETERMINANTS OF EMBRYO QUALITY
08 July 2020 Parallel 4 12:00 - 13:00

O-281 Complex mosaic embryos after preimplantation genetic testing: go for a second biopsy?

Z. Shuang[1], X. Pingyuan[2], H. Liang[3], T. Yueqiu[3], L. Ge[4]
[1]Hunan Guangxiu Hospital, Laboratory, Changsha- Hunan, China ;
[2]Hunan Normal University, School of Basic Medicine, Changsha- Hunan, China ;
[3]Central South University, Basic Medical College, Changsha- Hunan, China ;
[4]Reproductive and Genetic Hospital of Citic-Xiangya, Research Department, Changsha- Hunan, China

Study question: What is the incidence of complex mosaic in pre-implantation genetic testing (PGT) embryos and how to manage these embryos in clinical practice?
Summary answer: The incidence of complex mosaic blastocysts in PGT was 1.9%(253/13451) and it can be safe re-biopsy, implant and result in live births
What is known already: Some studies have reported that the births of babies after mosaic embryo transfer though the pregnancy rates were lower and the miscarriage rates were higher. The PGDIS and COGEN were given the practice recommendations on chromosome mosaicism, they were suggested if non-mosaic euploid embryos are not available, a non-complex, low-level mosaic embryo may be considered for transfer. But the complex mosaicism (mosaicism observed three or more chromosomes) were not recommended to transfer. The incidence of complex mosaicism appears to be lower, however, for the poor prognosis patients with only complex mosaic embryo, the clinical management becomes very complicated.
Study design, size, duration: Trophectoderm (TE) biopsy and NGS for genetic testing was performed on blastocysts generated from 13451 embryos between 2018 to 2019 in the IVF centre of the Reproductive and Genetic Hospital of CITIC-Xiangya. All biopsy samples were subjected whole genome amplification(WGA). The validated NGS platform were used to detect the copy number and the mosaic were defined 30-70%. The complex mosaicism were defined as three or more chromosomes with the copy number between 30% to 70%.
Participants/materials, setting, methods: Eighty-five patients opted to undergo warming, re-biopsy and re-vitrification in an effort to obtain a chance to transfer these embryos. Blastocysts resulting in a euploid diagnosis following the re-biopsy procedure were transferred with appropriate genetics counseling in subsequent frozen embryo transfer (FET) cycles. The main outcome measures were euploid, implantation and live birth rates. The study was approved by the ethics committee of the Reproductive and Genetic Hospital of CITIC-XIANGYA.
Main results and the role of chance: Overall, 13451 blastocysts were analyzed from 4359 PGT cycles, 253(1.9%) blastocysts from 243 cycles were complex mosaic. Of these 243 cycles with complex mosaic embryos, 85 patients opted to re-biopsy these embryos. The warmed rate were 96.7% (88 /91) and all the re-biopsied embryos were assigned a genetic diagnosis. Fifty-five re-biopsied blastocysts from a total 53 individual patients were diagnosed as euploid (55/88) and were suitable for subsequent FET. Of the 33 embryos that were abnormal, the most common diagnosis was mosaic, other abnormalities included 7 aneuploidy, 7 segmental aneuploidy, 2 complex mosaic. Sixteen patients have already undergone a subsequent FET of a re-biopsied single euploid blastocyst. The survival rate for blastocysts undergoing second warming was 100% (16/16) resulting in an ongoing pregnancy rate of 31.3% (5/16) and birth of two normal healthy babies to date.
Limitations, reasons for caution: But it should be noted that all the complex mosaic embryos re-biopsied in this study were with lower levels(30-50%) and the NGS platform for mosaic detection has been validated by known samples. At the time of writing the sample size is relatively modest.
Wider implications of the findings: The damage from laser, the quality of the TE cells, the bias in the WGA and NGS may be the reason for the complex mosaic in first biopsy samples. Finally, we all endeavor to maximize the most efficient route to a healthy birth for our patients in a single attempt.
Trial registration number: 81222007 and 31601183

O-282 Developmental potential and pregnancy outcomes of 210 mosaic embryo transfers - a single center experience

S. Madjunkova[1], M. Madjunkov[1], R. Antes[1], R. Abramov[1], S. Chen[1], H. Belakier[1], V. Kuznyetsov[1], C. Librach[1]
[1]Create Fertility Centre, Reproductive Genetics, Toronto, Canada

Study question: What is the developmental potential and pregnancy outcomes of mosaic embryos diagnosed by NGS based PGT-A and are there determinants that predict outcome?
Summary answer: Mosaic embryos have considerable implantation and developmental potential. Type and level of chromosomal aberrations did not impact implantation and ongoing pregnancy rates for mosaic embryos.
What is known already: Preimplantation genetic testing for aneuploidies(PGT-A) using NGS in IVF has increased rates of implantation per transfer, but at the same time has increased mosaic embryo detection to ~20%. Current recommendation is to consider mosaic embryos for transfer if there are no euploid embryos left. The evidence is limited on the developmental potential, implantation and birth outcomes of mosaic embryos. Some studies have suggested that level and type of chromosome aberrations may determine the implantation potential of mosaic embryos. This study aims to evaluate the implantation and pregnancy outcomes after mosaic embryo transfer detected at NGS resolution.
Study design, size, duration: This is a single centre retrospective cohort study where we analysed the clinical from 210 single mosaic embryo transfers from Jan. 2015-Dec. 2019.
Participants/materials, setting, methods: Clinical outcomes (implantation rate, ongoing pregnancy rate, miscarriage rate and birth outcomes) from 210 single mosaic embryo transfers from Jan. 2015-Dec. 2019 were available for analysis. NGS PGT-A analysis was performed using VeriSeq PGS (Illumina) kits. CNV analysis was done using BlueFuse software. The sensitivity for mosaicism detection was established at 20%, and aberrations considered clinically relevant were ≥10Mb in size and with ≥25% mosaicism.
Main results and the role of chance: Overall implantation rate for mosaic embryos was 53.3%. Of the 210 transferred mosaic embryos 80.9% had mosaic levels ≥25%-50% (Group 1) (58.6% with segmental mosaic (SM) gain or loss, 41.4% with whole chromosome mosaic (WCM) gain/loss) and 19.1% had mosaic levels of 50-70% (Group 2) (72.5% with SM and 27.5% with WCM). There was no statistical difference between Group 1 and Group 2 embryos in implantation rates (IR) 55.3% vs 45%, ongoing pregnancy rates (OPR) 46.5% vs 30%, and miscarriage rates (MR) 11.8% vs 10% respectively. Two pregnancies 2(1.2%) from Group 1 were ectopic. 70.9% of the ongoing pregnancies were from embryos diagnosed with SM (47.7% segmental losses, 28.4% segmental gains, 23.9% complex segmental gains/losses), while the rest 29.1% had whole chromosome aberrations-WCA (24% trisomies, 48% monosomies and 28% complex trisomy/monosomy). Similar distribution of SM and WCM were observed among miscarried embryos. There was no predictive outcome based on the chromosome involved in the SA mainly because of the small sample size. Birth outcome is available for 72 ET and healthy babies were delivered in all cases.
Limitations, reasons for caution: This is one of the largest studies presenting pregnancy outcomes after mosaic embryo transfer, however the sample size is still limiting the ability to correlate specific chromosomal aberrations with pregnancy outcomes. The sensitivity and specificity to detect mosaicism in our study applies to the assay used to diagnose mosaicism (NGS,VeriSeq-PGS-Kit).
Wider implications of the findings: Our findings provide evidence that mosaic embryos develop in healthy babies and support the hypothesis that low level mosaicism in early embryonic development may be a physiological phenomena. Our results will aid to better genetic counseling on the risks of mosaic embryo transfer, supporting their consideration for transfer.
Trial registration number: no trial registartion

O-283 Cumulus cells secrete microvesicles carrying miRNAs that might contribute to the acquisition of oocyte developmental competence

M. Zuccotti[1], D. Cimadomo[2], F. Cavalera[1], L. Dusi[3], S. Bertelle[3], B. Iussig[3], F. Ubaldi, Maria[2], L. Rienzi[2], S. Garagna[1]

[1]University of Pavia, Dipartimento di Biologia e Biotecnologie 'Lazzaro Spallanzani', Pavia, Italy ;
[2]Clinica Valle Giulia, Genera centers for reproductive medicine, Rome, Italy ;
[3]Genera Veneto, Genera centers for reproductive medicine, Marostica, Italy

Study question: What do the cumulus cells (CCs) secrete during the germinal-vesicle to metaphase II (GV-to-MII) transition that might mediate the acquisition of oocytes' developmental competence?
Summary answer: CCs release microvesicles which are internalised from the oocytes and contain miRNAs possibly involved in the acquisition of oocytes' developmental competence.
What is known already: During folliculogenesis oocytes grow and acquire developmental competence inside the cumulus-oocyte-complex (COC) thanks to a bidirectional communication with companion CCs. The nature of the CC-determinants contributing to oocytes' developmental competence remains poorly understood. We recently showed that when mouse CCs-free antral oocytes are cultured to MII with a feeder layer (FL) of CCs isolated from developmentally competent *surrounded-nucleolus* (FL-SN-CCs) or incompetent *not-surrounded-nucleolus* (FL-NSN-CCs) oocytes, the former acquire developmental competence to blastocyst, whereas the latter undergo developmental arrest. The hunt for FL-SN-CCs determinants points to the exocytosis of microvesicles containing functional molecules and to their fusion with or endocytosis by oocytes.
Study design, size, duration: Between March-2019 and December-2019, SN-FL-CCs and NSN-FL-CCs were prepared from CD1 mouse SN-COCs and NSN-COCs, respectively, to investigate the release of microvesicles into the culture medium and their internalisation into CCs-free mouse oocytes. The FL-CCs-derived microvesicles-specific miRNA content was screened from three cohorts of SN-FL-CCs and three of NSN-FL-CCs by RNA-sequencing. Sequencing was performed also on human FL-CCs produced between November-2018 and May-2019 from 6 IVF patients.
Participants/materials, setting, methods: Secreted-microvesicles were isolated by ultracentrifugation, sized and quantified with Nanosight. FL-SN-CCs were labelled with PKH67, co-cultured with CCs-free mouse antral oocytes and microvescicles internalization was assessed by confocal-microscopy. Next Generation RNA-sequencing, followed by bioinformatics analysis, was conducted to screen FL-CCs-derived microvesicles-specific miRNAs in i) mouse FL-SN-CCs versus FL-NSN-CCs cohorts, and ii) human FL-CCs obtained from 6 maternal age-matched cohorts of developmentally-competent (blastulation-rate per COC>30%, n=3) versus less-competent (blastulation-rate per COC<30%, n=3) oocytes.
Main results and the role of chance: After 15hr of maturation of mouse CCs-free antral oocytes upon FL-SN-CCs, a large number of microvesicles ($9.65x10^{10}$/ml) was detected in the media, 77% were potentially exosomes (129.7nm mean diameter and positive to Alix-targeted labelling).
Confocal-microscope analysis on three separate experiments showed that a massive number of CCs-derived microvesicles passed through the zona pellucida, while only a few could penetrate the ooplasm.

The comparison between the sequencing of miRNAs isolated from microvesicles released by mouse FL-SN-CCs versus FL-NSN-CCs highlighted 325 differentially expressed miRNAs, 21 of which up-regulated (top-5: mmu-miR-5112, mmu-let-7a-1-3p, mmu-let-7c-2-3p, mmu-miR-28c, mmu-miR-28a-5p) and 23 down-regulated (top-5: mmu-miR-712-5p, mmu-miR-1195, mmu-miR-410-3p, mmu-miR-122-5p, mmu-miR-342-5p). Pathway analysis (conducted with the DIANA miRpath v3 software using the micro-T-CDS database of predicted targets and the "genes union" merging algorithm) showed 27 and 42 pathways potentially controlled from the up-regulated and down-regulated miRNAs, respectively.

The comparison between miRNA sequencing data produced from microvesicles secreted by human CCs of developmentally-competent or less-competent cohorts of oocytes highlighted 370 miRNAs, 25 of which differentially expressed, 6 up-regulated (hsa-miR-3675-3p, hsa-miR-4741, hsa-miR-640, hsa-miR-133a-5p, hsa-miR-1909-3p, hsa-miR-6852-3p) and 19 down-regulated (top-5: hsa-miR-6866-5p, hsa-miR-1303, hsa-miR-7110-5p, hsa-miR-2278 and hsa-miR-4639-5p). The pathway analysis showed 12 and 8 pathways potentially controlled from the up-regulated and down-regulated miRNAs, respectively.
Limitations, reasons for caution: CCs-derived microvesicles internalization should be confirmed also in human oocytes. The sequencing data should be validated via more specific techniques, like qPCR and immunofluorescence, and with a larger samples size of CCs. Possibly, CCs should be isolated from single oocytes, rather than cohorts.
Wider implications of the findings: The identification of CCs-secreted microvesicles carrying miRNAs that are internalized by developing oocytes and might govern the acquisition of developmental competence will have numerous implications to improve: i) in-vitro-maturation, ii) treatment of patients suffering from reduced maturation rates (cancer, polycystic-ovarian-syndrome,

poor-ovarian-reserve and/or oocyte-maturation-arrest) iii) supplementation of culture media.
Trial registration number: not applicable

O-284 Abnormal fertilization in ICSI and association with abnormal semen parameters: A retrospective observational study on 1855 cases

A. Pappas[1], K. Pantos[1], K. Sfakianoudis[1], E. Maziotis[2], A. Rapani[2], E. Karantzali[2], S. Grigoriadis[2], P. Tsioulou[2], P. Giannelou[2], T. Vaxevanoglou[1], M. Chronopoulou[1], M. Koutsilieris[2], M. Simopoulou[2]

[1]Center for Human Reproduction, Genesis Athens Clinic, Athens, Greece ; [2]National and Kapodistrian University of Athens, Department of Physiology-Medical School, Athens, Greece

Study question: Is abnormal fertilization following ICSI related to abnormal semen analysis (SA) parameters?
Summary answer: A SA entailing more than two abnormal parameters-particularly for oligozoospermia-may compromise normal fertilization, cleavage and blastocyst formation rates, along with clinical pregnancy rates.
What is known already: Intracytoplasmic sperm injection (ICSI) efficiently addresses male factor infertility. However, even when ICSI is employed, a positive fertilization outcome may not be guaranteed. Undoubtedly, occurrence of abnormal fertilization patterns, mainly referring to abnormal pronuclei (PN) patterns of extra or missing pronuclei, merits further investigation. This may be heightened especially with regards to management of couples presenting with a trend for abnormal fertilization patterns. Identifying potential causative associations between specific abnormal SA parameters, their respective combination, and fertilization outcome following ICSI may be of added value for the IVF laboratory.
Study design, size, duration: This retrospective observational study included 1855 ICSI cycles performed between 2014 and 2018. Medical records from couples submitted to a first fresh autologous ICSI cycle between 2014 and 2019 were retrieved and included in the study. The principal inclusion criteria were male age of 18-50 years old, female age of the partner 18-40 years old, and employment of the gonadotropin-releasing hormone (GnRH) long agonist stimulation protocol.
Participants/materials, setting, methods: The participants were divided into groups, according to pathologies identified following SA, and the respective combinations of individual abnormal parameters, based on WHO criteria. Cases of normal SA served as the control group. Presence of two PN and extrusion of the second polar body indicated normal fertilization. Embryo transfer was performed on day three or five, based on the number and quality of cleavage stage embryos. R programming language was used for the statistical analysis.
Main results and the role of chance: From the total of 1855 men, 162 presented without any abnormal observations in the SA, serving as the control group. Two-hundred and ninety-five were diagnosed with asthenozoospermia, 229 with oligozoospermia, 83 with teratozoospermia, 232 with oligoasthenozoospermia, 213 with oligoteratozoospermia, 284 with asthenoteratozoospermia and 356 with oligoasthenoteratozoospermia (OAT). The results demonstrated that SA pathologies affect 2PN formation rate ($p<0.0001$), fertilization failure ($p=0.0001$), cleavage rate ($p=0.0001$) and blastocyst formation rate ($p<0.0001$). No correlation was established between abnormalities and 1PN or 3PN formation rates. Regarding 2PN rate, the control group presented with the highest (0.82±0.05) and the OAT group presented with the lowest (0.60±0.08). The highest fertilization failure rates were reported in the oligoasthenoteratozoospermic (0.246±0.0749) and the lowest in the normal group (0.155±0.0511). The lowest cleavage rates were identified for oligoasthenozoospermic and oligoasthenoteratozoospermic patients (0.814±0.508 and 0.78±0.0999). The aforementioned groups along with oligoteratozoospermic patients presented with the lowest blastocyst formation rate (0.457±0.043, 0.453±0.068 and 0.459±0.035). Regarding clinical pregnancy data, only oligoasthenozoospermic and oligoasthenoteratozoospermic patients were associated with lower rates compared to the normal group (OR:0.538, 99.3225%CI:0.302-0.956; p-value=0.004 and OR:0.582, 99.3225%CI: 0.344-0.985; p-value=0.005, respectively). Following adjustment for number of blastocysts no statistically significant difference was observed regarding the clinical pregnancy rate.
Limitations, reasons for caution: The retrospective nature of this observational study may be correlated with bias, along with the fact that the study was conducted in a single center. Finally, DNA fragmentation index assessment is not included herein, albeit representing a valuable complimentary tool to evaluate sperm quality.
Wider implications of the findings: Different abnormal SA parameters may lead to different IVF cycle outcomes. Oligozoospermic semen samples featuring at least one more abnormal parameter are associated with compromised blastocyst formation rates. Oligoasthenozoospermic and OAT patients present with lower clinical pregnancy rates. Depending on SA parameters a different IVF treatment strategy may be required.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 69: BIOMARKERS OF DEVELOPMENTAL COMPETENCE

08 July 2020 Parallel 1 14:00 - 15:15

O-285 An oocyte assessment tool using machine learning; Predicting blastocyst development based on a single image of an oocyte

D. Nayot[1], J. Meriano[2], R. Casper[1], K. Alex[3]

[1]TRIO Fertility, Reproductive Endocrinology and Infertility, Toronto, Canada ; [2]TRIO Fertility, Embryology, Toronto, Canada ; [3]FutureFertility, Embryology, Toronto, Canada

Study question: Can an Artificial Intelligence (AI) based image analysis tool predict the blastocyst development potential of oocytes better than the current standard?
Summary answer: Our AI image analysis tool outperformed all 17 embryologists by an average of 21.2% in predicting blastocyst development and, unlike the embryologists, was 100% reproducible.
What is known already: There are no morphological features of oocytes that have been validated to have prognostic value for further developmental competence (Rienzi 2011). Currently there is no standardized or accepted visual oocyte scoring system (Alpha 2011), and therefore limited feedback about oocyte quality is available to patients and clinicians.

Deep learning offers promise for the automation and standardization of embryo quality assessment. There are several studies using machine learning in an attempt to automate embryo grading and improve embryo selection, but this is the first clinical application focusing on oocytes.
Study design, size, duration: The Violet (an AI image analysis tool) was created with convoluted neural networks based on a retrospective dataset (n=17,659 oocyte images). It's able to predict fertilization and blastocyst development with 91.2% and 63% accuracy respectively in an unbalanced dataset.

In a balanced test set of 300 oocytes, the Violet outperformed all 17 embryologists, from 8 IVF clinics, in accurately predicting fertilization (71.7% vs 58.9±4.3%; 21.8% increase) and blastocyst development (62.8% vs 52.2±3.7%; 20.2% increase).
Participants/materials, setting, methods: N/A
Main results and the role of chance: In an unbalanced data set, the Violet was able to predict fertilization and blastocyst development with 91.2% and 63% accuracy respectively. It was especially effective at identifying negative cases with 99% accuracy when the confidence was > 70%

Two validation studies were performed to compare the Violet to embryologists. A balanced data set of 300 oocyte images was randomly selected from the test subset: 100 failed fertilization, 100 fertilized but did not reach blastocyst stage, and 100 reached blastocyst stage.

The Violet outperformed all 17 embryologists, from 8 IVF clinics, in accurately predicting fertilization (71.7% vs 58.9% ± 4.3%; 21.8% increase) and blastocyst development (62.8% vs 52.2% ± 3.7%; 20.2% increase).

In a reproducibility study, 7 of these embryologists underwent the same task 2-3 months later. The average accuracy remained close to chance, 53% ± 3.3% with an intra-observer reproducibility of 81.4% for blastocyst formation, while the Violet was 100% reproducible.

Limitations, reasons for caution: As with all AI image analysis tools, a larger and more diverse data set is necessary to extrapolate findings. A prospective multi-centre validation study is currently underway to validate the Violet technology
Wider implications of the findings: The lack of a visual oocyte assessment criteria is congruent with our findings that 17 senior embryologists were essentially unable to predict blastocyst development (average 52.2%). Machine learning image analysis improves our ability to assess oocyte quality in an instantaneous, non-invasive, reproducible and more accurate method than the current standard.
Trial registration number: 16325-16:10:0722

O-286 Fresh or vitrified oocytes from the same donor cohort: do they differentially affect clinical outcomes?

J. Maidana[1], E. De Martino[1], M. Papayannis[1], P. Filardi[1], C. Bisioli[1], G. Terrado[2], I. De Zúñiga[2], M. Horton[2], M. Bianchi[2], H. Pettorossi[2], N. Passi[2], M. Marcelli[2], F. Sobral[2], M. Gómez Peña[1]

[1]Pregna Reproductive Medicine, Embryology Laboratory, Buenos Aires, Argentina ; [2]Pregna Reproductive Medicine, Reproductive Medicine, Buenos Aires, Argentina

Study question: Do clinical outcomes differ when using fresh or vitrified oocytes from the same donor cohort?
Summary answer: Fresh or vitrified oocytes lead to similar clinical outcomes in an egg donation program.
What is known already: The extended use of frozen oocytes in egg donation programs rest on the success of oocyte vitrification and the need to optimise synchronisation between donors and recipients. Oocyte vitrification has proven its efficacy, however the impact of thermal and osmotic stress on embryo development and clinical results still remains controversial.
Study design, size, duration: We retrospectively compared embryo development (fertilisation, embryo quality and blastocyst formation) and clinical outcomes (implantation, pregnancy and miscarriage rates) of fresh and vitrified oocytes cycles from the same cohort of 34 donors in 117 recipients between January 2017 and December 2018.
Participants/materials, setting, methods: Recipient cycles were divided into two groups: those who received fresh (n= 75) or vitrified oocytes (n= 42). All oocytes (n= 679) were fertilised by ICSI and transfers were performed at the blastocyst stage. Cycles with frozen embryo transfers or severe male factor were excluded from this study. The mean number of embryos transferred was similar in both groups (1.01 vs 1.0). Chi-Square test was used as statistical data analysis.
Main results and the role of chance: Each recipient received 5.4 and 6.5 MII oocytes on average in the synchronous (406 fresh) and the asynchronous group (273 vitrified) respectively (p=NS). A total of 323 oocytes were warmed and 273 (84.5%) survived. Although blastocysts formation rate (58.1 vs 48.4%, p<0.05) and the rate of usable blastocysts (45.5 vs 35.6%, p<0.05) were higher in the fresh oocyte group, no differences were observed between fresh and vitrified oocytes regarding fertilisation rate (80.5 vs 80.2%; p=NS), percentage of good quality embryos (80.0 vs 86.0%; p=NS), clinical pregnancy (55.0 vs 45.0%; p=NS), miscarriage (2.0 vs 10.0%; p=NS) or implantation rates (55.0 vs 50.0%;p=NS).
Limitations, reasons for caution: Our study is limited by its sample size and retrospective design. Oocytes were donated by healthy, young women so these results should not be generalised to other populations.
Wider implications of the findings: Although blastocyst formation was significantly lower in the group of vitrified oocytes, we didn't observe differences in clinical outcomes. This could mean a possible stress effect or cell pre-conditioning for better stress tolerance. Our study suggests that the reproductive results should not be affected by donor oocyte vitrification.
Trial registration number: Not applicable

O-287 F-actin and MYO10 protein distribution: a novel tool in assessing the quality of human growing follicles

A. Volodarsky-Perel[1], S. Granados Aparici[2], T. Tulandi[1], W. Buckett[1], S. Krishnamurthy[1], F. Williamson Mansour[1], J. Papillon Smith[1], H. Clarke[1]

[1]McGill University, Obstetrics and Gynecology, Montreal, Canada ; [2]McGill University, Research Institute, Montreal, Canada

Study question: Does the distribution of F-actin and MYO10 protein differ in human preantral follicles from patients of different age and ovarian pathology?
Summary answer: Advanced age and endometriosis have an impact on the distribution pattern of F-actin in granulosa cells and MYO10 protein in granulosa cells and oocytes.
What is known already: Direct markers of oocyte quality described so far include spindle dysfunction and chromosomal misalignment during meiotic maturation, whereas markers of the surrounding granulosa cells usually focus on cell proliferation and death. Granulosa cells communicate with the oocyte via actin-rich cytoplasmic extensions termed transzonal projections (TZPs) which penetrate the zona pellucida and enable the oocyte to be supplied with essential nutrients and regulatory signals necessary for its growth. MYO10 protein likely regulates filamentous (F-) actin assembly or function in TZPs. However, the expression and distribution pattern of MYO10 protein and F-actin in women of different ages and clinical conditions remains unclear.
Study design, size, duration: A prospective study was conducted among 21 women who underwent ovarian surgery and consented to fresh ovarian tissue donation in the period from 2017 to 2019. Ovarian follicles of primordial to secondary stages were retrieved from 9 women aged 22 to 40 years. Four had no ovarian pathology; the other five were diagnosed with endometriosis confirmed by histological analysis.
Participants/materials, setting, methods: Fresh tissue samples were immediately delivered for follicle harvesting. Follicles with a morphologically normal oocyte and non-pyknotic granulosa cells were fixed and stained using phalloidin and anti-MYO10. Confocal microscopy was used to image equatorial optical sections of each follicle. Parameters measured were the number of MYO10 foci and intensity of F-actin in the granulosa cells and oocytes. Correlation between the oocyte diameter and the structures associated with TZP development (MYO10 foci and F-actin) was evaluated.
Main results and the role of chance: A total of 93 good-quality follicles were included in the analysis. Mean oocyte diameter was 32.6 ± 6.8 µm. A correlation between oocyte diameter and MYO10 distribution (correlation estimate (CE) 0.37; 95% confidence interval (95% CI) 0.08-0.61; P = 0.01) as well as between oocyte diameter and F-actin intensity (CE 0.36; 95% CI 0.07-0.59; P = 0.008) in granulosa cells was demonstrated in patients of 22-30 years of age. These correlations for both MYO10 distribution and F-actin intensity with oocyte diameter were not observed in the group of 35-40 years (P = 0.15, P = 0.16, respectively). The intensity of F-actin in granulosa cells was also correlated with oocyte diameter in patients with normal ovaries (CE 0.31; 95%CI 0.02-0.55; P = 0.02). This correlation was not observed in patients with endometriosis (P = 0.83). Strikingly large MYO10 aggregates within the oocyte were significantly more common in cases with endometriosis compared to those with normal ovaries (14 (41.2%) versus 8 (17.8%); P = 0.02). The absence of correlation of MYO10 and F-actin distribution with oocyte diameter suggests a disorganization of TZPs development in small growing follicles, which may contribute to poorer follicle quality in older women and patients with endometriosis.
Limitations, reasons for caution: Only fresh ovarian samples were included in the study. The effect of age and ovarian pathology on the structures associated with TZP development in frozen ovarian tissue is a matter for further studies.
Wider implications of the findings: We suggest that MYO10 distribution and F-actin intensity in granulosa cells as well as the presence of MYO10 aggregates in oocytes can be used for quality assessment of ovarian follicles in women of different ages and ovarian pathology.
Trial registration number: not applicable

O-288 Dynamic oxygen level (5%-2%) during human in vitro embryo culture significantly improves usable blastulation and cumulative live birth rates in in vitro fertilization (IVF)

S. Brouillet[1], A. Andreeva[1], A. Gala[1], A. Ferrieres[1], M. Anav[1], A. Fournier[1], V. Loup[2], N. Ranisavljevic[2], S. Hamamah[1]

[1]University of Montpellier - University hospital of Montpellier - INSERM U1203, preimplantation genetic testing- assisted reproductive techniques- CECOS, Montpellier, France ; [2]University hospital of Montpellier, preimplantation genetic testing- assisted reproductive techniques- CECOS, Montpellier, France

Study question: Does dynamic oxygen level (5-2%) during *in vitro* embryo culture improve IVF outcomes compared to continuous oxygen level (5%)?

Summary answer: Dynamic oxygen level significantly increases total and usable blastocyst formation rates as well as cumulative implantation, clinical pregnancy and live birth rates in IVF.

What is known already: In IVF, human embryos are usually cultured under 20% or 5% of oxygen. A dynamic oxygen exposure during *in vitro* culture may represent the most physiologic system as *in vivo* oxygen tension is ≈5% for early cleaved embryos in the oviduct and ≈2% for morulas/blastocysts in the uterus. Moreover, ultra-low oxygen tension (2%) increases the expression of antioxidant enzymes in mouse embryos and enhances the proliferation rate of human trophoblast, protecting the blastocyst against an excess in oxidative stress during its high metabolic activity and providing a favorable environment for blastocyst viability and implantation potential.

Study design, size, duration: A monocentric retrospective cohort study was performed from June 2014 to March 2019. A total of 120 couples were enrolled. The study was approved by local institutional review board (2019_IRB-MTP_05-12). All couples underwent one IVF cycle associated with continuous oxygen exposure (5% from Days 0-6) and the subsequent IVF cycle associated with dynamic oxygen exposure (5% from Days 0-3 and 2% from Days 3-6) for *in vitro* embryo culture.

Participants/materials, setting, methods: The first objective was to evaluate total and usable blastocyst formation rates in both "continuous 5% oxygen exposure" and "dynamic 5-2% oxygen exposure" groups. Blastocysts were scored using the Gardner grading system. The secondary objective was to evaluate cumulative implantation, clinical pregnancy and live birth rates obtained after fresh and frozen-thawed morula/blastocyst transfers in both "continuous 5% oxygen exposure" and "dynamic 5-2% oxygen exposure" groups.

Main results and the role of chance: The maternal age (35.3 ± 4.4 vs. 33.5 ± 4.6, p=0.003) and the total number of IVF cycles (2.7 ± 1.0 vs. 1.6 ± 0.9, p< 0.0001) were significantly higher in the "dynamic 5-2% oxygen exposure" group. All other clinical and biological parameters were similar in both groups. The blastocyst formation rate (54.8% vs. 44.4%, p=0.0007) and usable blastocyst formation rate (32.8% vs. 21.8%, p<0.0001) were both significantly higher in the "dynamic 5-2% oxygen exposure" group than in "continuous 5% oxygen exposure" group. Implantation, clinical pregnancy and live birth rates seem increased in the "dynamic 5-2% oxygen exposure" group compared to the "continuous 5% oxygen exposure" group after fresh morula/blastocyst transfers, but the differences didn't reach significance. The cumulative implantation (33.1% vs. 16.8%, p=0.016), clinical pregnancy (36.8% vs. 15.2%, p=0.006), and live birth (23.2% vs. 8.4%, p=0.004) rates were significantly higher in the "dynamic 5-2% oxygen exposure" group than in "continuous 5% oxygen exposure" group, respectively.

Limitations, reasons for caution: This is a retrospective study. The selection of embryos to be transferred and cryopreserved was based on embryo morphology criteria that might be different in other clinics.

Wider implications of the findings: Improved IVF outcomes were obtained in the same couples when dynamic oxygen exposure (5-2%) was used compared to continuous oxygen exposure (5%), in spite of the negative impact of increased maternal age. Dynamic oxygen exposure (5-2%) may improve blastocyst formation and implantation potential by preventing oxidative stress during IVF.

Trial registration number: 2019_IRB-MTP_05-12

O-289 Detection of Cannabis in Follicular Fluid and its impact on Oocyte Quality, Endocannabinoid Signaling, and Epigenetics

N. Fuchs Weizman[1], B. Wyse[2], P. Szaraz[3], M. Defer[2], S. Jahangiri[4], C. Librach[5]

[1]University of Toronto, Department of Obstetrics, Toronto, Canada ; [2]CReATe Fertility Centre, Research - Ovarian Biology Group, Toronto, Canada ; [3]CReATe Fertility Centre, Research - Cardiac Stem Cell Group, Toronto, Canada ; [4]CReATe Fertility Centre, CReATe BioBank, Toronto, Canada ; [5]CReATe Fertility Centre, Medical Director, Toronto, Canada

Study question: How do phytocannabinoids (PC) in follicular fluid (FF) affect the dynamics of endocannabinoid receptors (endoCB-Rs) and of methyl-transferase expression in the surrounding granulosa cells?

Summary answer: Marijuana exposure does appear not alter the dynamics of the endocannabinoid system; however, it changes the epigenetic machinery in the follicular niche.

What is known already: THC, a cannabis plant derivative, is used for medicinal and recreational purposes. It is the third most commonly used substance by women of childbearing age, hence knowledge of the effect it has on reproduction is of utmost importance. THC exerts its effects via receptors of the endocannabinoid system and can interfere with its regular functions which are required for successful reproduction. Moreover, previous studies have shown THC alters methylation and histone modifications in sperm, brain, and blood cells. The levels of endocannabinoids have been measured in follicular fluid obtained during oocyte retrieval and are implicated in controlling folliculogenesis.

Study design, size, duration: Samples were previously biobanked from consenting patients during IVF oocyte retrieval. Their follicular fluid was analyzed for patient exposure to phytocannabinoids, and to measure endoCBs. The corresponding granulosa cells (hGC) were utilized to explore the dynamics of endoCB-Rs and a methylating enzyme in the follicular niche. Cases were patients with at least one PC detected in their follicular fluid, and matched controls were negative for all three tested PCs (delta9-THC, 11-OH-delta9-THC, 11-COOH-delta9-THC).

Participants/materials, setting, methods: We performed liquid chromatography-mass-spectrometry (LC-MS/MS) at the Analytical Facility for Bioactive Molecules (SickKids Hospital, Toronto, CA) on FF obtained from dominant follicles between Jan 2018-July 2019. Cases were matched with controls by age, BMI, AMH and trigger day E2 levels. Levels of endoCB-Rs (CB1R-APC, CB2R-Alexa488; R&D Systems) and of the methylating enzyme DNMT3b (DNMT3b-APC, Miltenyi Biotec), in corresponding hGC were assessed by flow cytometry (MACSQuant 10). Median fluorescence intensities were calculated (FlowJo10).

Main results and the role of chance: Out of 244 samples analyzed, 17 tested positive for PCs (6.5%) and these were matched with 15 samples that were negative for all PCs tested (controls). The percentage of positive samples among our population increased from 3.8% to 13.3% after legalization of cannabis in Canada in October 2018 (chi^2; p=0.02). The concentrations of PCs did not change following legalization. There was no significant effect of PCs on maturation rate (mean case maturation rate 75.9% vs. control maturation rate 74.4%). In addition, the levels of endocannabinoids (including AEA, 2-AG, among others (10 in total)) did not differ significantly between cases and controls. Next, the cell surface protein expression levels of endoCB-Rs in corresponding hGC was examined. Overall CB2R expression was higher than CB1R, however, exposure to PCs did not significantly alter this expression. Taken together, this indicates that PC exposure might not alter endocannabinoid signaling significantly. DNMT3b, a DNA methylation enzyme, is involved in de-novo methylation and is crucial for epigenetic integrity. Interestingly, exposure of hGC to PC decreased DNMT3b positive events, indicating that cannabis exposure appears to affect epigenetic machinery at the protein level.

Limitations, reasons for caution: Our study is limited by lack of details regarding mode, frequency, and timing of PC consumption. A further limitation is the small sample size, which warrants larger-scale studies to validate our findings. Finally, future studies should also focus on the effects of PC exposure on developing oocytes.

Wider implications of the findings: To our knowledge, this is the first study measuring cannabis derivatives in FF by LC-MS/MS. We show that consuming cannabis does not appear to affect the endocannabinoid system in the developing follicle. Lastly, we have implicated cannabis in disrupting epigenetic mechanisms in hGCs.

Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 70: OVARIAN STIMULATION STRATEGIES IN IVF AND IUI

08 July 2020 Parallel 2 14:00 - 15:15

O-290 Impact of controlled ovarian stimulation on vaginal and endometrial microbiota in IVF cycles: a pilot study

A.R. Carosso[1], A. Revelli[1], G. Gennarelli[1], S. Canosa[1], S. Cesarano[1], N. Brunod[1], C. Scarafia[1], S. Leoncini[1], L. Boatti[2], E. Zanotto[3], F. Sidoti[3], R. Cavallo[3], C. Benedetto[1]

[1]Obstetrics and Gynecology 1U- Physiopathology of Reproduction and IVF Unit, Department of Surgical Sciences- Sant'Anna Hospital- University of Torino, torino, Italy ;

[2]University of Piemonte Orientale, SmartSeq s.r.l.-, Novara, Italy ;
[3]Division of Virology- University of Turin- Città della Salute e della Scienza di Torino, Public Health and Pediatrics Department, Turin, Italy

Study question: Does controlled ovarian stimulation (COS) modify the vaginal and endometrial microbiota of women undergoing in vitro fertilization (IVF)?
Summary answer: COS modifies vaginal and endometrial microbiota inducing a reduction of Lactobacillus abundance and an increased heterogeneity, leading to the potential growth of pathogenic bacteria.
What is known already: COS is associated with raising estrogen levels and several studies report improved pregnancy rate when frozen embryos transfers are performed, probably due to a better endometrial receptivity. Although changes of the vaginal microbiota depending on hormone levels during a menstrual cycle have been demonstrated, the impact of raising estrogens levels associated with COS on the female microbiota is unknown. Furthermore, the effects of the presence of an active vaginal and endometrial microbiota on the outcomes of IVF treatments are still controversial.
Study design, size, duration: In this pilot study, 15 women (age, 29-42 years) undergoing IVF or ICSI treatment attending the Reproductive Physiopathology Center of the S. Anna Hospital in Turin (Italy) were included between July 2018 and October 2019.
Participants/materials, setting, methods: Patients underwent vaginal and endometrial microbiota analysis in two moments:

- PRE-COS ANALYSIS: simulation of embryo transfer plus a vaginal swab were performed in the luteal phase of the cycle preceding COS.
- POST-COS ANALYSIS: at the time of fresh embryo(s) transfer.

The distal extremity of the catheter tip and the vaginal swab were analyzed by 16S rRNA gene sequencing. To characterize the samples, the Shannon diversity index (SDI) was used.
Main results and the role of chance: The genus most present in the PRE-COS vaginal samples was that of Lactobacillus, as in POST-COS analysis, although with lower abundance (71.5 ± 40.6% and 61.1 ± 44.2 %, respectively). At the same time, an increase of pathogenic species, such as Prevotella (3.5 ± 8.9% Vs 12 ± 19.4%) and Atopobium (5.7 ± 10.6% Vs 5.6 ± 9.4%), was observed. In the endometrium, the genus most represented was that of Lactobacillus, although the concentrations decreased between the two analyses (27.4 ± 34.5 % and 25 ± 29.9 %, PRE-COS and POST-COS respectively), with a simultaneous increase of Prevotella and Atopobium (3.4 ± 9.5% Vs 4.7 ± 7.4 % and 0.7 ± 1.5 Vs 5.8 ± 12 %). The Shannon indices evaluated at the PRE-COS and POST-COS analysis were significantly different, indicating an effect of COS on vaginal and endometrial microbiota diversity ($p< 0.001$ for both sites). Furthermore, the presence of a Lactobacillus dominated microbiota at POST-COS analysis in the endometrium was associated with a greater thickness at ultrasound monitoring on the day of ovulation trigger (12 ± 0.8 mm Vs 8.8 ± 0.9 mm, $p<0.005$). However, an impact on pregnancy rates of vaginal and endometrial microbiota was not observed.
Limitations, reasons for caution: Our research is a pilot study and it is limited by the restricted number of patients included. Furthermore, the presence of bacteria in the endometrium forming an active microbiota is still discussed.
Wider implications of the findings: Our results indicate that vaginal and endometrial microbiota undergo relevant modification after COS. COS induces higher instability at both levels that could justify the negative reproductive and obstetric outcomes of a proportion of IVF treatments supporting the diffusion of the "freeze all strategy".
Trial registration number: not applicable

O-291 Ovarian stimulation strategies in intrauterine insemination for unexplained or mild male factor infertility – an individual participant data meta-analysis

R. Wang[1], N.A. Danhof[2], M.P. Diamond[3], R.S. Legro[4], P. Karen[5], M. Erdem[6], T. Dankert[7], E. Rene[8], F. Van der Veen[2], B.W. Mol[1], M. Van Wely[2], M.H. Mochtar[2], *. On behalf of the IUI IPDMA Collaboration[2]

[1]Monash University, Department of Obstetrics and Gynaecology, Clayton, Australia ;
[2]Amsterdam UMC, Centre for Reproductive Medicine, Amsterdam, The Netherlands ;
[3]Augusta University, Department of Obstetrics and Gynecology, Augusta, U.S.A. ;
[4]Penn State College of Medicine, Department of Obstetrics and Gynecology, Hershey, U.S.A. ;
[5]UZ Leuven, Leuven University Fertility Center, Leuven, Belgium ;
[6]Gazi University Faculty of Medicine, Department of Obstetrics & Gynecology, Ankara, Turkey ;
[7]Rijnstate Hospital, Department of Obstetrics and Gynecology, Arnhem, The Netherlands ;
[8]Hospices Civils de Lyon, Service de Biostatistique-Bioinformatique, Lyon, France

Study question: Does ovarian stimulation with gonadotrophin or letrozole increase live birth rate compared to clomiphene citrate (CC) in couples with unexplained or mild male factor infertility undergoing intrauterine insemination (IUI)?
Summary answer: Ovarian stimulation with gonadotropins results in higher live birth rate compared to CC in couples undergoing IUI while evidence on letrozole versus CC is insufficient.
What is known already: Ovarian stimulation in IUI aims to increase pregnancy rates by increasing the number of dominant follicles. Ovarian stimulation can be achieved with CC, letrozole and gonadotropins. Today IUI with ovarian stimulation is the main source of multiple pregnancies. Multiple pregnancies carry the risk of higher morbidity to mother and child. Individual participant data meta-analysis (IPD-MA) is considered as the gold standard for evidence synthesis which provides accurate assessments of outcomes from primary randomised controlled trials (RCTs) and allows additional analyses for time-to-event outcomes.
Study design, size, duration: We performed an IPD-MA of relevant RCTs. We searched PubMed, MEDLINE, EMBASE, CENTRAL and the Clinical Trial Registration Database indexed up to 16 August 2018. No language restrictions were applied.
Participants/materials, setting, methods: We included RCTs that compared ovarian stimulation with CC, letrozole or gonadotrophins to each other in IUI among couples with unexplained infertility or mild male factor infertility. We contacted the authors of the identified trials to join and share their IPD. The primary effectiveness outcome was live birth and the primary safety outcome was multiple pregnancy. The secondary outcomes included clinical pregnancy and time to ongoing pregnancy leading to live birth.
Main results and the role of chance: We identified 22 eligible RCTs (4624 couples). Authors from 6 RCTs provided IPD. After further excluding 411 couples with infertility due to anovulation or other factors, we included 2,299 couples in this IPD-MA.

Compared to IUI with CC, IUI with gonadotrophins increased live birth rate (RR 1.28, 95%CI 1.06-1.55, 5 RCTs) and clinical pregnancy rate (RR 1.22, 95%CI 1.06-1.40, 6 RCTs), while it reduced time to ongoing pregnancy leading to live birth (HR 1.38, 95%CI 1.15-1.66, 5 RCTs). Data between the two groups on multiple pregnancy were inconclusive (RR 1.55. 95%CI 0.57-4.22, 5 RCTs). RCTs with IPD and RCTs without IPD showed similar results in this comparison. One RCT compared letrozole versus CC in IUI. The evidence on IUI with letrozole versus CC was inconclusive on live birth (RR 0.80, 95%CI 0.59-1.10), clinical pregnancy (RR 0.79, 95%CI 0.60-1.04), multiple pregnancy (RR 1.13, 95%CI 0.44-2.89) and time to ongoing pregnancy leading to live birth (HR 0.78, 95%CI 0.55-1.11). RCTs with IPD and RCTs without IPD showed inconsistent results on clinical pregnancy (group difference p=0.01) and none of the RCTs without IPD reported live birth in this comparison.
Limitations, reasons for caution: The evidence between letrozole and CC are based on one RCT sharing the IPD. The difference between RCTs with and without IPD may be due to the differences in study population or study quality.
Wider implications of the findings: Ovarian stimulation with gonadotrophins should be considered as the first-line treatment in couples with unexplained or mild male infertility undergoing IUI, if intracycle monitor is available and extra costs are acceptable. There is an urgent need for further RCTs to compare letrozole and CC in IUI to confirm existing evidence.
Trial registration number: CRD42017053966

O-292 In vitro maturation versus in vitro fertilization in women with high antral follicle count: a cost-effectiveness analysis alongside a randomised clinical trial

H. Vu Ngoc Anh[1,2], N. Loc Minh Tai[1,2], H. Tuong Manh[1,2], D. Vinh Quang[1,2], W. Rui[3], N. Robert J[4], M. Ben[3], V. Lan Ngoc[1,2,5]

[1]IVFMD, My Duc Hospital, Ho Chi Minh City, Vietnam ;
[2]HOPE Research Center, My Duc Hospital, Ho Chi Minh, Vietnam ;
[3]Monash University, Department of OB/GYN, Melbourne, Australia ;
[4]Robinson Research Institute, University of Adelaide, Adelaide, Australia ;
[5]University of Medicine and Pharmacy at HCM City, Department of OB/GYN, Ho Chi Minh, Vietnam

Study question: Is in vitro maturation (IVM) more cost-effective than in vitro fertilization (IVF) in women with high antral follicle count (AFC)?
Summary answer: IVM should be considered as cost-effective if society is willing to accept €8,847 for foregoing an additional live birth.
What is known already: Both IVF and IVM are treatment options in women with high AFC undergoing ART, mainly anovulatory women not responding to ovulation induction. IVM has no risk of ovarian hyperstimulation syndrome (OHSS) and is potentially more patient friendly. However, the cost-effectiveness of IVM versus IVF has not been assessed.
Study design, size, duration: This cost-effectiveness analysis was based on data from a randomised clinical trial at IVFMD, My Duc Hospital, HCMC, Vietnam (NCT03405701). Between December 2017 to April 2019, women were randomly assigned to one cycle of IVM or one cycle of IVF at IVFMD. The effectiveness measure for the CEA was the cumulative live birth rate after one completed cycle including subsequent cryo-cycles within 12 months following randomisation.
Participants/materials, setting, methods: We collected data on resource use related to treatment, medication, pregnancy from case report forms. The primary economic analysis was performed from a health care perspective. We measured costs relating to treatment (medication, complications, pregnancy, delivery). We additionally did the analysis from a societal perspective including non-medical costs. We calculated mean costs, effects, averaged differences in costs and incremental cost-effectiveness ratios (ICER) using nonparametric bootstrap resampling to assess the effect of uncertainty in our estimates.
Main results and the role of chance: A total number of 546 women were randomized to the IVM group (n = 273) or the IVF group (n = 273). Cumulative live birth rates after one completed cycle were 112/273 (41.0%) in the IVM group vs. 160/273 (58.6%) in the IVF group (RR 0.70, 95%CI 0.59-0.83). OHSS did not occur in the IVM group versus 2/273 (0.7%) in the IVF group. The mean total costs per couple were €5,684 for IVM and €7,240 for IVF (Absolute difference €-1,556, 95%CI €-1,837 to €-1,274). The ICER for IVM compared with IVF per additional live birth was €8,847 (95%CI €8,634 - €9,083).
Limitations, reasons for caution: We only compared one cycle of IVM versus one cycle of IVF. As data were collected from a single centre, unit costs obtained might be less representative in other settings.
Wider implications of the findings: IVM is less effective, but less expensive than IVF, therefore, the use of IVM in women with high AFC depends on society's willingness to accept foregoing an additional live birth.
Trial registration number: NCT03405701

O-293 Evaluation of Time Interval Between Ovulation Trigger With Triptorelin Acetate and Oocyte Retrieval in IVF Cycles. A Randomized Controlled Trial

C. Díaz-García[1], N. Garcia Camuñas[2], A. Marzal[3], M.J. Soriano[4], J. Subira[4], A. Garcia belda[4], A. Pellicer[5]

[1]IVI London, Reproductive Medicine, London, United Kingdom ;
[2]Hospital Universitario La Fe, IVF Unit, Valencia, Spain ;
[3]IVI Valencia, Reproductive medicine, Valencia, Spain ;
[4]IIS La Fe, Reproductive Medicine Research Group, Valencia, Spain ;
[5]IVI Roma, Reproductive Medicine, Rome, Italy

Study question: What is the optimal time interval between GnRH agonist trigger and follicular aspiration to maximise the number of mature oocytes collected?
Summary answer: The egg collection performed after 36 hours of triptorelin acetate administration allows obtaining a higher number of metaphase II (MII) oocytes
What is known already: Both GnRH agonists (GnRHa) and hCG have proven to be effective in achieving ultimate oocyte maturation. Nevertheless, their mechanism of action is different, and the hormonal dynamics of the ovulatory peak and the molecular profiles differ between them. GnRHa faithfully reproduces the physiological characteristics of natural cycles. Therefore, the accurate time to achieve oocyte maturation could be different between the two drugs. Consequently, the optimum moment to perform the follicular puncture after the GnRHa administration could be different from the 36 hours described for hCG. A study of the optimum time interval between ovulation triggering and follicular puncture is crucial
Study design, size, duration: Randomised, controlled, single-blind clinical trial carried out in a university-based IVF unit between September 2014 and December 2017. Patients undergoing ovarian stimulation with FSH in a short antagonist protocol were randomized to undergo egg collection after 30, 36 or 40 hours after ovulation trigger with 0.2 mg of triptorelin acetate. In patients undergoing fresh embryo transfer, luteal phase support was achieved by administering 1500 IU of hCG and 400 mg of vaginal micronized progesterone
Participants/materials, setting, methods: Women aged 18-37 years with a baseline FSH <10mIU/ml, AMH 5-45pmol/l, AFC 6-24 were included in the study. Patients with at least 5 follicles ≥16mm were randomized. Egg collections were done separating follicles measuring more and less than 16mm. Embryo transfer was performed according to usual clinical practice. The main outcome variable was the number of MII oocytes retrieved and the number of MII oocytes retrieved per follicle measuring > 16mm the day of trigger
Main results and the role of chance: A total of 121 patients were randomized to undergo ovulation triggering and egg collection after 30h (n=41), 36h (n= 42) or 40 hours (n=38). The proportion of MII retrieved was significantly lower after 30h of Decapeptyl administration in comparison with 36h and 40h follicular aspiration times (30h: 6.63±4.14, 36h: 9.10±4.75 and 40h: 7.26±4.49; p=0.03698). In addition to that, the ratio MII/follicles >16mm was higher 36h after trigger, compared to the 30h group (0.98±0.56 vs 0.73±0.39; p=0.05277). MII rates were concordant with molecular analysis results: a significantly down-regulation of genes related to oocyte maturation (AREG, BTC, CYP19A1, EFNB2, EREG, PHLDA1, RGS2 and UGP2) were found in GCs of punctured follicles at 30h. No significant differences regarding pregnancy rates or live birth rates were found between groups
Limitations, reasons for caution: The external validity of this study could be limited to patients undergoing the same type of ovarian stimulation. The present study was not designed to detect differences in pregnancy or live birth rates. Larger studies need to be performed in order to confirm the findings of the present study
Wider implications of the findings: The results of this study contribute to improving IVF protocols in order to recover the maximum number of MII oocytes and therefore, enhancing pregnancy rates. In addition, the use of GnRHa for ovulation induction would decrease the risk of OHSS associated with hCG stimulation protocols
Trial registration number: NCT02244151

O-294 Duration of infertility and IVF outcomes: analysis of 252 359 IVF cycles

Y. Beebeejaun[1], T. Copeland[2], I. Sarris[1], M. Kamath[3], S. Sunkara[4]

[1]King's Fertility, Women's Health, London, United Kingdom ;
[2]University of California Los Angeles, Department of Health Policy &-Management-, Los Angeles, U.S.A. ;
[3]Christian Medical College-, Department of Reproductive Medicine, Vellore, India ;
[4]King's College London, Women's Health, London, United Kingdom

Study question: Does infertility duration affect clinical and perinatal outcomes following IVF treatment?
Summary answer: The present study demonstrated an increased risk of low birth weight when the duration of infertility exceeds 48 months. Further research validation is warranted.
What is known already: Infertility is associated with adverse reproductive outcomes and women with infertility have a higher risk of adverse perinatal

outcomes. Furthermore, risk of adverse perinatal outcomes is also higher following assisted reproductive treatments (ART) compared to spontaneous conceptions. Globally, when IVF is indicated for infertility, the duration of infertility varies when women undergo treatment. There are several factors that influence when women have IVF. These factors could be other failed treatments before resorting to IVF, logistical or funding issues. With scant literature on whether infertility length could affect IVF outcomes, it is a matter of interest to explore this.

Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of ART in the UK.

Data from 1991 to 2016 comprising a total of 252 493 first fresh IVF ± ICSI cycles were analysed based on the length of infertility (< 24 months vs 24 to 48 months vs > 48 months). Multiple births were excluded for analysis of perinatal outcomes.

Participants/materials, setting, methods: Perinatal outcomes of pre-term birth (PTB), early PTB (ePTB) and low birth weight (LBW) and very LBW (vLBW) as well as clinical outcomes of live birth rate, miscarriage and stillbirth were analysed in singleton live births. Occurrence of a live birth at < 37 weeks gestation is defined as a PTB and at < 32 weeks gestation as ePTB. Birth weight < 2500 grams is defined as LBW and < 1500 grams as vLBW.

Main results and the role of chance: Logistic regression analysis was performed adjusting for female age category, previous pregnancy, period of treatment, cause of infertility (male factor, tubal disease, ovulatory disorder, endometriosis, unexplained) and number of oocytes retrieved. Our control group was those suffering from infertility for <24 months.

Clinical outcomes: The live birth rate following single embryo transfer was 25.8% (95% CI 25.6%- 26.2%) for those suffering from infertility for less than 24 months, 24.6% (95% CI 24.3%-25.0%) for those suffering from infertility between 24 and 48 months and 21.9% (95% 21.6%-22.2%CI) when the duration of infertility is over 48 months. The relative risk ratio (RRR) of miscarriage was 0.94 (p-value=0.09) and 1.03 (p-value=0.35) when the duration of infertility was between 24-48 months and >48 months respectively The RRR of stillbirth was 0.87 (p-value=0.44) and 0.88 (p-value=0.11) for the 24-48 months and > 48 months period respectively.

Perinatal outcomes: The relative risk of ePTB and vLBW was not seen to be associated with increased duration of infertility. However, the RRR of PTB was 1.01 (p-value=0.84) and 1.11 (p-value=0.04) for the 24-48 months and >48 months respectively. , For LBW, the RRR was 0.99 (p-value=0.78 in the 24-48 months and 1.13 (p-value= 0.01) in the >48 months cohort respectively.

Limitations, reasons for caution: Limitations with observational data would apply to this study including residual confounding. This is the first study to address this study question.

Wider implications of the findings: The perinatal outcomes of offsprings born following ART techniques is of clinical relevance for patients and clinicians.

Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 71: ABOUT HOW SPERM QUALITY AND MALE INFERTILITY RELATE TO GENETICS

08 July 2020 Parallel 3 14:00 - 15:15

O-295 The influence of poor semen parameters on embryonic chromosome segregation

E. Fragouli[1,2], D. Babariya[1], G. Cutts[1], V. Lozoya Garcia[3], E. Fernandez Marcos[1], L. Parnell[1], A. Symon-Allen[1], F. Bronet[4], M. Florensa[5], N. Prados[6], A. Mercader[7], N. Garrido[3], D. Wells[1,2]

[1]IVI-RMA Global, Juno Genetics, Oxford, United Kingdom ;
[2]University of Oxford, Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom ;
[3]IVI-RMA Global, IVI Foundation, Valencia, Spain ;
[4]IVI-RMA Global, IVI Madrid, Madrid, Spain ;
[5]IVI-RMA Global, IVI Barcelona, Barcelona, Spain ;
[6]IVI-RMA Global, IVI Seville, Seville, Spain ;
[7]IVI-RMA Global, IVI Valencia, Valencia, Spain

Study question: Is male factor infertility associated with an altered incidence of chromosomal abnormalities of potential relevance to subsequent preimplantation development?

Summary answer: Male factor infertility is associated with increased mosaic chromosome abnormalities, a potential consequence of the sperm's role in formation of the first mitotic spindle.

What is known already: Infertility affects one in six couples worldwide with male factors contributing in ~50% of cases. Currently, the relative insensitivity of methods used to assess the competence of male gametes represents a significant limitation in evaluating the likelihood that a sperm sample will produce viable embryos if used for oocyte fertilisation. The close relationship between advancing female age and increasing oocyte aneuploidy rates has been well described. However, the extent to which the male gamete influences the genetics of the early embryo and its subsequent development is less clear.

Study design, size, duration: 331 embryos produced by couples with male factor (MF) infertility (semen sample concentration <5million/ml with/without testicular biopsy or epididymal aspiration) were evaluated, and their cytogenetic status retrospectively compared to that of 1245 embryos derived from infertile couples without MF evidence. Examination involved trophectoderm (TE) biopsy followed by next generation sequencing (NGS) for preimplantation genetic testing for aneuploidy (PGT-A). NGS enabled the accurate identification of mosaic and non-mosaic aneuploidies in blastocysts generated by both patient groups.

Participants/materials, setting, methods: 517 couples having IVF with PGT-A in 12 clinics participated. 69 patients (average female age 38.2 years) underwent PGT-A due to MF infertility. The remaining 448 (average female age 40.2 years) underwent PGT-A due to other indications. Patients were divided into "younger" (MF- 32.8 years, others-33.7 years average female age) and "older" (MF- 39.7 years, others- 40.6 years average female age) for data comparison. A highly validated targeted NGS approach was employed for chromosomal analysis.

Main results and the role of chance: More MF generated blastocysts were euploid compared with those generated by patients undergoing PGT-A for other indications (49% versus 32%, P<0.001). This is presumably a consequence of lower meiotic error rates in the oocytes of MF couples, due to their lower average female age. Conversely, MF patients generated significantly (P<0.001) more mosaic blastocysts (38%) compared with the remaining patients [17% 95%CI (2.906)], a difference that was detectable in both age groups. This increased mosaicism incidence suggests that the MF generated embryos experience more mitotic errors post-fertilisation. No significant difference (P=0.118) in the incidence of segmental aneuploidies was observed between the two patient groups. Of the 339 abnormalities scored in MF generated blastocysts, 286 (84%) affected whole chromosomes and 53 (16%) affected segments. A total of 1853 aneuploidies were identified in the blastocysts generated by the patients undergoing PGT-A for other indications, and 1624 (88%) affected whole chromosomes, with the remaining 229 (12%) being partial. This finding argues against sperm DNA fragmentation having a possibly detrimental effect after fertilisation. MF infertility patients had a significantly lower no-transfer rate, compared to all remaining patients (16% vs. 39% respectively, P<0.001), presumably a consequence of the higher incidence of euploid embryos in this group.

Limitations, reasons for caution: Classification was based on TE samples biopsied from blastocysts during PGT-A. As only a fraction of the cells from each embryo are assessed, some mosaic embryos may be incorrectly classified as fully euploid or aneuploid. However, this misclassification is expected to have little impact on the overall conclusions.

Wider implications of the findings: Poor sperm parameter patients are predisposed to generate mosaic embryos. This predisposition should be discussed during the counselling of couples considering PGT-A due to MF. Sperm centrioles are responsible for the first mitotic spindle organisation. Impaired centriole function in suboptimal sperm might explain the increased post-fertilisation chromosome segregation error rates.

Trial registration number: Not applicable

O-296 Single-cell genome-wide bisulfite sequencing for assessing the role of DNA methylation in spermatozoa.

"Abstract withdrawn by the authors"

O-297 Genetic and Epigenetic Characterization of Globozoospermic Men to Tailor Assisted Reproductive Treatment

D. Tavares[1], A. Parrella[1], S. Cheung[1], P. Xie[1], Z. Rosenwaks[1], G. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Can we genomically characterize globozoospermia using various biomarkers and nucleic acid sequencing to determine the best reproductive treatment?

Summary answer: Globozoospermia is associated with specific gene mutations and imbalances. Bioassays can be used to assess this gamete's capacity to support embryonic development and tailor treatment.

What is known already: Round-headed spermatozoa lack an acrosome and have a defect in chromatin compaction, primarily diagnosed by transmission electron microscopy (TEM). The phenotype of globozoospermia is mostly related to an autosomal recessive mutation and is frequently associated with familial inheritance. While few globozoospermia genotypes have been identified, fortunately, this rare defect does not always have complete penetrance. We applied different bioassays and genomic studies to better characterize this gamete's capacity to support embryonic development, as well as to tailor treatments to achieve pregnancy.

Study design, size, duration: Semen parameters, sperm ultrastructural details, protamine content, sperm chromatin fragmentation (SCF), sperm cytosolic factor, and sperm aneuploidy were assessed for 12 globozoospermic men. The genome and transcriptome of 3 men were profiled and compared to specimens from donors with proven fertility. ICSI was performed with or without assisted gamete treatment (AGT) based on diagnostic results of the bioassays. Clinical outcomes of conventional ICSI cycles and subsequent ICSI cycles with AGT were compared.

Participants/materials, setting, methods: Semen analysis was performed on 12 patients. TEM surveyed acrosomal defects and centrosomal integrity. Aniline Blue assay quantified protamine content with ≤20% normal threshold. SCF was measured by TUNEL with ≤15% normal threshold. Sperm cytosolic factor was determined by PLCζ assay with ≥30% normal threshold. Sperm aneuploidy was assessed using FISH with <1.6% normal threshold. Genome and transcriptome were analyzed by NGS and RNA-seq, respectively. AGT was performed by exposing both gametes to calcium ionophore.

Main results and the role of chance: Globozoospermia was defined by the presence of round-headed spermatozoa exceeding 70%. Twelve globozoospermic men (35.8±5 years) had a concentration of 34.4±44x106/ml, 24±25% motility, and normal morphology of 0.1±0.3%. Concurrent testing revealed abnormal protamine content of 53.8±24%, elevated SCF of 19.4±2%, insufficient cytosolic factor of 6.0±4%, and a high aneuploidy rate of 4.4±5%. Complete globozoospermia was confirmed in 3 men by TEM and the absence of PLCζ; genomic analysis identified 2 gene mutations: DPY19L2 and SPATA16. Epigenetic analysis revealed 142 differentially expressed genes compared to the control. We also found an overexpression of both mutant DPY19L2 and SPATA16 genes. To characterize their clinical outcomes, 7 couples (men, 35.8±5 years; women, 32.8±5 years) underwent 22 ICSI cycles yielding a fertilization rate of 31% (85/274), an implantation rate of 13.6% (6/44), and a clinical pregnancy rate (CPR) of 22.2% (4/18), with 1 pregnancy loss. For the remaining 3 couples with complete globozoospermia (men, 37.3±5 years; women, 36.6±6 years), historical ICSI cycles (n=3) yielded a 44% (8/18) fertilization rate, but no implantation. Once treated by AGT (n=4), there was a significant improvement, with a 50% fertilization rate (12/24) (P<0.00001), a 22% implantation rate (2/9), and a 33.3% CPR (1/3), resulting in 1 live birth.

Limitations, reasons for caution: This is the first study that attempts to utilize different bioassays and characterize the genome and epigenome of globozoospermic men in order to tailor reproductive treatment. While this preliminary data is reassuring, the health of the resulting offspring should be investigated.

Wider implications of the findings: The limitations of semen analysis are more apparent in cases of globozoospermia. This study demonstrates the association of this rare teratozoospermia to gene structure and function. Ultrastructural assays and genomic studies can be used to characterize this gamete's capacity to support embryonic development and to tailor treatments maximizing reproductive outcome.

Trial registration number: not applicable

O-298 Average sperm head area: a novel risk factor for sperm aneuploidy in idiopathic male infertility

M. Ruiz - Jorro[1], M. Ferrer-Buitrago[2], J.J. Bataller-Sánchez[3], A. Barberá-Alberola[3], X. Vendrell-Montón[4], C. Calatayud-Lliso[5]

[1]CREA Medicina de la Reproducción SL, Reproductive Andrology, Valencia, Spain ; [2]CREA. Medicine de la Reproducción SL, Research and Development, Valencia, Spain ; [3]CREA Medicina de la Reproducción SL, Andrology Laboratory, Valencia, Spain ; [4]Sistemas Genómicos. Grupo Biomédico Ascires, Reproductive genetics, Paterna, Spain ; [5]CREA Medicina de la Reproducción SL, Reproductive Medicine, Valencia, Spain

Study question: Is increased average of sperm head area (ASHA) a risk factor of sperm aneuploidy in patients consulting for infertility?

Summary answer: Increased ASHA values are associated with sperm aneuploidy in patients in whom altered meiosis during spermiogenesis is not initially suspected.

What is known already: Sperm aneuploidy test is aimed at patients experiencing recurrent implantation failure (RIF) or pregnancy loss (RPL). Likewise, patients with high FSH levels due to a testicular failure are prone to accumulate meiotic errors during spermiogenesis. Although sperm aneuploidy is detected in up to 15 % of infertile men, the risk of chromosomal aberrations increases to 40% in patients with factors such as RIF, RPL or suspected testicular failure. Fluorescence *in situ* hybridation (FISH) is employed to analyze sperm aneuploidy. This costly test is usually requested after observing repeated reproductive failures. Finding early indications to assess sperm aneuploidy remains a challenge.

Study design, size, duration: To determine the cut-off value for ASHA, we initially analyzed a total of 142 patients who showed normal FISH results (control group). The positive predictive value of ASHA was further assessed in a first retrospective study, which included a total of 250 patients experiencing RIF, RPL or testicular failure (Group 1). The validation of ASHA was assessed in a total of 22 patients who did not refer RIF, RPL or testicular failure (Group 2).

Participants/materials, setting, methods: Patients enrolled in CREA IVF program participated in the present study, after signing an informed consent form. ASHA cut-off value was established according to the value at percentile 95. Two semen samples were analyzed per patient according to WHO-V-manual. Sperm parameters, such as: sperm concentration, motility, morphology and ASHA were evaluated using the CASMA commercial software ISAS. Samples were stained employing the Diff-quick kit. FISH analysis (5 chromosomes) was performed using Metafer-4 software.

Main results and the role of chance: ASHA cut-off value was established as ≥14,8 μm^2 in the control group. Group 1 revealed an incidence of altered FISH of 41,2%. Furthermore, ASHA showed lower variability amongst the studied samples (p<0,002) and higher positive predictive value to detect altered FISH (73,9%) than other sperm parameters such as concentration (56,5%), total sperm count (58,8%) and morphology (46,7%). To reduce the potential bias associated with the retrospective data (patients with suspected risk of sperm aneuploidy), the predictive value of ASHA was further validated in a prospective analysis. Sample size was adjusted to detect at least 30 % difference from the basal 15 % incidence reported in a global population of infertile patients. All patients in Group 2 showed normal testicular function, with FSH values lower than 10 mUI/ml (4,81 ± 2,83). Sperm parameters in group 2 were: concentration (20,79 ± 26,47 million sperm/ ml), motility (44,95 ± 14,58) and morphology (1,55 ± 0,74%). The mean ASHA value in group 2 was 15,22 ± 0,64, with an incidence of sperm aneuploidy of 59,09%. This prospective analysis reveals that patients with ASHA values > 14,8 μm^2 show a higher incidence of chromosomal aberrations in sperm than a global population of infertile men (p<0,05).

Limitations, reasons for caution: The present study evaluates a limited number of patients. Further analysis in a broader population of patients with idiopathic infertility is needed to confirm the present findings.

Wider implications of the findings: ASHA is a sperm parameter which indicates the risk of sperm aneuploidy from the first sperm analysis. Its assessment contributes to reduce the time to advise PGT-A, and consequently the time to conceive in some infertile couples. Our findings provide novel insights in the field of androgenetics and male infertility.

Trial registration number: Not applicable

O-299 Attributing ICSI Fertilization Failure to the Responsible Gamete

P. Chung[1], S. Cheung[1], P. Xie[1], A. Parrella[1], D. Keating[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Is there a way to identify and effectively treat the gamete responsible for complete fertilization failure (CFF) with ICSI?

Summary answer: Screening the male gamete for the presence of PLCζ may identify the specific gamete responsible for ICSI CFF and guide treatment in subsequent cycles.

What is known already: ICSI is capable of overcoming defects of the zona pellucida and sperm acrosomal dysfunction, allowing fertilization. However, although rare, CFF with ICSI can occur due to oocyte activation deficiencies, either oocyte-related or due to the absence of sperm-activating factors. Most treatment approaches of CFF with ICSI described in the literature involve different methods of oocyte activation (AOA), yielding inconsistent results. These approaches may overlook cases of CFF that occur despite apparently normal gamete characteristics and often due to ooplasmic dysmaturity.

Study design, size, duration: Over 20 years, couples with ≤10% ICSI fertilization were identified. Seventy-six men were screened for the presence of PLCζ. Couples with oocyte-related OAD (n=52) underwent subsequent ICSI with tailored stimulation. Those with sperm-related OAD (n=24) were further screened by mouse oocyte activation test (MOAT) before undergoing subsequent ICSI with assisted gamete treatment (AGT). Consenting men (n=4) underwent a genomic evaluation by NGS. ICSI outcome was compared between treatment and historical cycles of the same couple.

Participants/materials, setting, methods: A total of 114 couples, with female partners ≤37 years of age and male partners with a sperm concentration of ≥1x10^6/ml, had fertilization rates of ≤10% despite injecting ≥3 oocytes (IRB 0712009553). PLCζ assessment was performed, confirmed by MOAT and DNA/RNA sequencing. In subsequent cycles, couples with oocyte-related OAD were treated by modulating *in-vivo/vitro* maturation time, while sperm-related OAD cases were treated by exposing both gametes to calcium ionophore. Clinical outcomes were compared.

Main results and the role of chance: A total of 114 couples (maternal age, 33.8±4 yrs; paternal age, 36.9±5 yrs) with a 9.1% ICSI fertilization rate were identified. Consenting male partners were screened, and 52 couples were identified as having oocyte-related OAD. These couples underwent subsequent ICSI cycles with tailored superovulation, yielding significantly higher fertilization (59.0% vs. 2.1%; *P*<0.0001) and clinical pregnancy (28.6% vs. 0%; *P*<0.0001) rates. Thirty clinical pregnancies resulted in 25 deliveries and 5 pregnancy losses. A total of 32 babies were born, 15 boys and 17 girls, with no major or minor congenital malformations.

Twenty-four couples (maternal age, 35.6±5 years; paternal age, 39.8±6 years) were confirmed as having sperm-related OAD following PLCζ and MOAT tests. In some men, DNAseq showed a PLCZ1 deletion, corroborating the initial screening. Deletions of genes associated with spermiogenesis and embryo development (PIWIL1, BSX, NLRP5), and the absence of subacrosomal perinuclear theca (PICK1, SPATA16, DPY19L), were also found. In these couples, AGT treatment provided higher fertilization (42.1% vs. 9.1%; *P*<0.05) and clinical pregnancy (36% vs. 0%; *P*<0.05) rates compared to the historical cycles. Six patients have successfully delivered, with offspring displaying normal development at 3 years of age.

Limitations, reasons for caution: In this prospective cohort study, while we controlled for maternal age-related aneuploidy, confounding factors cannot be excluded with certainty. Although PLCζ and MOAT assays need further validation, they serve an important purpose in identifying gamete-specific causes of CFF and in preventing the overuse of calcium ionophore treatment.

Wider implications of the findings: This is the first attempt to identify a gamete-specific OAD in couples with ICSI CFF. Those with an oocyte-related OAD can be treated in a subsequent cycle with a modified superovulation protocol, preventing AGT overuse. For those with sperm-related OAD, genomic analysis helped to identify the related genes involved.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 72: PREGNANCY LOSS: WHAT TO CONSIDER

08 July 2020 Parallel 4 14:00 - 15:15

O-300 Results of a highly significant, prematurely halted Dutch multicenter randomized double-blinded placebo-controlled study of pretreatment with mifepristone to misoprostol in early pregnancy failure (triple m)

L. Hamel[1,2], J. Van den Berg[1], S. Coppus[3], M. Snijders[2], F. Vandenbussche[4]

[1]Radboud University Medical Centre, Obstetrics and Gynaecology, Nijmegen, The Netherlands ;
[2]Canisius-Wilhelmina Hospital, Obstetrics and Gynaecology, Nijmegen, The Netherlands ;
[3]Maxima Medical Centre, Obstetrics and Gynaecology, Veldhoven, The Netherlands ;
[4]Kreiskrankenhaus Mechernich GmbH, Obstetrics and Gynaecology, Mechernich, Germany

Study question: Is, in early pregnancy failure (EPF), the sequential combination of mifepristone with misoprostol superior to misoprostol alone in terms of complete evacuation of the uterus?

Summary answer: In early pregnancy failure, the sequential combination of mifepristone with misoprostol is highly superior to misoprostol alone in reaching complete evacuation of the uterus.

What is known already: In case of EPF, women can choose from either expectant, medical or surgical management. Surgical intervention carries a risk of complications, however medical treatment appears to be a safe alternative. When the Dutch common practice of at least one week of expectant management is adhered to, current medical treatment with misoprostol alone has complete evacuation rates just above 50%. Shortly after the start of this trial, Schreiber et al (NEJM 2018) reported in a non-blinded trial that pretreatment with Mifepristone appears to be more successful when treating instantaneously after EPF is diagnosed.

Study design, size, duration: This study is a multicenter, randomized, double-blinded, placebo-controlled trial executed at 18 hospitals in the Netherlands. Results of the pre-planned interim-analysis, performed after 50% (N=232) of patients were enrolled, led to a premature halt of the study, due to highly significant outcomes. The study started on June 27th 2018, the 232nd inclusion was achieved in September 2019. When the decision was made to stop this trial prematurely, in total 342 woman had been randomized.

Participants/materials, setting, methods: Women with a diagnosis of EPF between 6-14 weeks gestation were included after at least one week of expectant management. They were randomized between pretreatment with mifepristone 600mg or placebo, both followed by two doses of misoprostol 400µg orally on day three and four. Ultrasonography was performed 15-20 days after treatment and, if required, repeated 4 weeks later to assess successful treatment, defined as absence of gestational sac in utero and endometrial thickness <15 mm.

Main results and the role of chance: After performing an interim-analysis of 50% of the pre-calculated required number of patients (n=232), as stated in advance in the study protocol, complete evacuation of the uterus was found to be 77,1% in the mifepristone group versus 52,9% in the placebo group (p=0.000). After 6 weeks of follow-up, the need for curettage was 10,2% in the mifepristone group compared to 31,9 % in the placebo group (p=0.000). Because these results met the predefined stopping rule, the Data Safety Monitoring Board instructed us to stop the study, since the difference between the two groups had already been proven beyond doubt, in favor of pretreatment with mifepristone. There were no concerns regarding the safety of the patients enrolled in the study. At the ESHRE 2020 annual meeting, follow-up for all 342 randomized women will be completed, and a full analysis of the treatment effect can be reported.

Limitations, reasons for caution: There is no consensus about the definition of 'successful treatment', or the optimal treatment regimen for EPF regarding dose or routes of administration of medical treatment for EPF.

Wider implications of the findings: In addition to adjustment of the Dutch national guideline concerning EPF, these results contribute to the improvement of medical management of EPF around the world. Especially in low income countries, this will lead to an extensive improvement of both the effectiveness and accessibility of proper medical treatment of EPF.
Trial registration number: NCT03212352

O-301 Reproductive performance following application of hyaluronic acid gel after dilatation and curettage for miscarriage in women with at least one previous curettage

A. Hooker[1], R. De Leeuw[2], J. Twisk[3], J. Huirne[2]

[1]Zaans Medical Center ZMC, Department of Obstetrics and Gynaecology, Zaandam, The Netherlands ;
[2]Amsterdam UMC- location VU University Medical Center- Amsterdam- the Netherlands., Department of Obstetrics and Gynecology-, Amsterdam, The Netherlands ;
[3]Amsterdam UMC- location VU University Medical Center- Amsterdam- the Netherlands., Department of Epidemiology and Biostatistics, Amsterdam, The Netherlands

Study question: Does intrauterine application of auto-crosslinked hyaluronic acid (ACP) gel, following dilatation and curettage (D&C) improve reproductive outcome in women with at least one previous D&C.
Summary answer: Application of ACP gel after D&C for miscarriage in women with at least one previous D&C.seems to have a favorable effect on subsequent reproductive outcome
What is known already: Application of auto-crosslinked hyaluronic acid (ACP) gel, in women who experienced at least one previous D&C undergoing D&C for miscarriage, resulted in a significant lower rate of IUAs, 13.0% versus 30.6%, relative risk (RR) 0.43 (95% CI 0.22-0.83, *P*=.013, lower mean adhesion score and significant less moderate to severe IUAs. Although subsequent fertility is a key outcome, data on reproductive performance following application of ACP after D&C for miscarriage remain limited.
Study design, size, duration: This was a follow-up study of a prospective randomized trial, conducted in one university and seven university-affiliated teaching hospitals in the Netherlands. Women with a miscarriage of < 14 weeks with at least one previous D&C, were randomized to D&C plus ACP gel (intervention) or D&C alone (control). A hysteroscopy was performed after 8-12 weeks to detect intrauterine adhesion (IUAs). In case IUAs were encountered, adhesiolysis was executed.
Participants/materials, setting, methods: To evaluate reproductive outcomes, participants of the PAPA-study were approached between December 2017 and April 2018. Participants (n=139) received questionnaires by post or email at least 30 months after randomization. The questionnaires consisted of 45 items related to received interventions, complications, menstrual pattern, contraceptive use, the desire to become pregnant, conception and outcome of subsequent pregnancies. The primary outcome was ongoing pregnancy.
Main results and the role of chance: The response rate in the intervention group was 93.1% and in the control group 95.5%. Baseline characteristics were comparable in both groups. The median duration of follow-up was slightly longer in the intervention group, 40.6 months (SD 2.7) versus 39.2 months (SD 3.6) in the control group. Ongoing pregnancies were recorded in 74.6% in the intervention group versus 67.2% in the control group, OR 1.33 (0.63-2.82). The miscarriages rate was 20.9% versus 37.5% respectively, OR, 0.44 (95% CI 0.18-0.90, *P*=.05). The live birth, ectopic pregnancy and TOP rates were not significantly different. In women wishing to conceive, ongoing pregnancies were recorded in 94.3% versus 71.7% , OR 4.84 (1.51-15.51) and more live births, 86.8% versus 70.0% in the control group OR, 2.82 (95% CI 1.07-7.42; *P*=.04) The median time to conception leading to a live birth was 21.9 versus 36.1 months, hazard ratio 1.46 (0.96-2.48).
Limitations, reasons for caution: The main limitation is that the PAPA-study was designed and powered for the presence of IUAs and not for long-term reproductive outcomes. Therefor the outcomes should be interpreted with caution.
Wider implications of the findings: The current study shows that application of ACP seems to have a favorable effect on reproductive performance. Our results should be confirmed in a large prospective study of sufficient power. .
Trial registration number: NTR 3120

O-302 First contact at implantation in humans: Endometrial epithelium induces trophectoderm differentiation to invasive syncytiotrophoblast.

P. Ruane[1], L. Parsons[1], T. Garner[1], P. Babbington[1], S. Kimber[2], A. Stevens[1], M. Westwood[1], D. Brison[1], J. Aplin[1]

[1]University of Manchester, Maternal and Fetal Health, Manchester, United Kingdom ;
[2]University of Manchester, Division of Cell Matrix Biology and Regenerative Medicine, Manchester, United Kingdom

Study question: How does the human embryo breach the endometrial epithelium at implantation?
Summary answer: Embryo attachment to the endometrial epithelium induces the formation of multinuclear syncytiotrophoblast from trophectoderm, which goes on to breach the epithelial layer.
What is known already: A significant proportion of assisted reproduction treatments fail due to unsuccessful implantation. The trophectoderm of the blastocyst-stage embryo attaches to the endometrial epithelium before breaching this barrier to implant into the endometrium. While historical histology suggested invasive syncytiotrophoblast after the embryo has breached the epithelium, *in vitro* models are required to understand the processes of human embryo implantation.
Study design, size, duration: Fresh and frozen human blastocyst-stage embryos (n=46) and human trophoblast stem cells were co-cultured with an endometrial epithelial cell line to model implantation *in vitro*. Systems biology approaches with published transcriptomic datasets were used to model implantation *in silico*.
Participants/materials, setting, methods: Human embryos surplus to treatment requirements were consented for research. Day 6 blastocysts were co-cultured with Ishikawa endometrial epithelial cell line monolayers until day 8, and trophoblast stem cell spheroids were co-cultured for 48h. Morphology was assessed by immunofluorescence microscopy, and trophoblast differentiation by RT-qPCR and ELISA. Human blastocyst, trophoblast stem cell and endometrial organoid transcriptomic datasets were used with hypernetwork analysis and random forest machine learning to identify gene networks associated with implantation.
Main results and the role of chance: The majority of embryos (37/46, 80.4%) breached the Ishikawa cell layer, and syncytiotrophoblast was observed in all of these. Sites where a single point of breaching had occurred always consisted of syncytiotrophoblast (7/7). Multiple (up to 7), independent syncytiotrophoblast regions were seen in 26/46 (56.5%) embryos. Human trophoblast stem cell spheroids co-cultured with Ishikawa layers also exhibited synscytiotrophoblast formation upon breaching the epithelium. RT-qPCR demonstrated epithelium-induced upregulation of syncytiotrophoblast genes CGB (p=0.03), OVOL1 (p=0.04) and SDC1 (p=0.008), and ELISA revealed stimulation of hCG secretion (p=0.03). Receptive endometrial organoid surface transcriptome was used to identify trophectoderm surface binding partners and hypernetwork analysis established a group of 24 trophectoderm receptors that were highly connected within the transcriptome. Of these, 14 genes are differentially expressed in trophoblast stem cell-syncytiotrophoblast differentiation ($p<0.047$-8.7^{-9}), and random forest machine learning assigned 20/24 genes to processes driving this differentiation pathway ($p<0.01$). *In vitro* embryo and stem cell models, and *in silico* models, all suggest that syncytiotrophoblast formation from trophectoderm is induced by attachment to the endometrial epithelium and this cell type pioneers embryo invasion.
Limitations, reasons for caution: *In vitro* and *in silico* models may not recapitulate the dynamic interactions embryo-endometrial interactions that occur *in vivo*. The influence of other cellular compartments in the endometrium, including decidual stromal cells and leukocytes, was not recapitulated in these models.
Wider implications of the findings: Understanding the mechanism of human embryo breaching of the epithelium and the gene networks involved is crucial to improve implantation success rates after assisted reproduction. Moreover, early trophoblast lineages form the blueprint for the placenta and thus underpin fetal growth trajectories, pregnancy health and even offspring health.
Trial registration number: NA

O-303 Comparison of DNA methylation patterns of parentally imprinted genes in placenta derived from IVF conceptions in two different culture media

C. Mulder[1], T. Wattimury[1], A. Jongejan[2], C. De Winter-Korver[1], S. Van Daalen[1], R. Struijk[1], S. Borgman[1], Y. Wurth[3], D. Consten[3], J. Van Echten-Arends[4], S. Mastenbroek[1], J. Dumoulin[5], S. Repping[1], A. Van Pelt[1], A. Van Montfoort[5]

[1]Amsterdam UMC - Location AMC, Center for Reproductive Medicine- Amsterdam Reproduction and Development Research Institute ., Amsterdam, The Netherlands ;
[2]Amsterdam UMC- location AMC, Bioinformatics Laboratory- Clinical Epidemiology- Biostatistics and Bioinformatics- Amsterdam Public Health Research Institute- Amsterdam UMC, Amsterdam, The Netherlands ;
[3]St. Elisabeth Hospital, Center for Reproductive Medicine, Tilburg, The Netherlands ;
[4]University Medical Center Groningen, Section of Reproductive Medicine, Groningen, The Netherlands ;
[5]Maastricht University Medical Center, Department of Obstetrics & Gynaecology- GROW School for Oncology and Developmental Biology, Maastricht, The Netherlands

Study question: Is there a difference in DNA methylation status of imprinted genes in IVF derived placentas where embryo culture was performed in HTF versus G5 medium?
Summary answer: We found no statistically significant differences in the mean DNA methylation of imprinted genes in placentas derived from IVF conceptions cultured in HTF versus G5.
What is known already: Animal studies indicate that the embryo culture environment affects the DNA methylation status of the embryo. In humans, birthweight is known to be affected by the type of embryo culture medium used. The effect of embryo culture media on pregnancy, birth and child development may thus be mediated by differential methylation of parentally imprinted genes in the placenta.
Study design, size, duration: DNA methylation status of imprinted genes was determined in human placenta derived from IVF conceptions exposed to HTF or G5 embryo culture medium. Placenta samples (n=43 for HTF, n=54 for G5) were collected between 2010 and 2012 as part of a multi-centre randomized controlled trial in the Netherland comparing these embryo culture media. Placenta samples from 69 naturally conceived live births collected during 2008-2013 in the Netherlands were used as reference material.
Participants/materials, setting, methods: To measure the DNA methylation status of differentially methylation regions (DMRs) associated with parentally imprinted genes, we opted for an amplicon-based sequencing strategy on an Illumina MiSeq sequencing platform. DNA was isolated and 34 differentially methylated regions associated with well-defined parentally imprinted genes, were amplified in a two-step PCR procedure before sequencing using MiSeq technology. Sequencing data were analysed in a multivariate fashion to eliminate possible confounding effects.
Main results and the role of chance: We found no statistically significant differences in the mean DNA methylation status in any of the imprinted DMRs in placentas derived from IVF conceptions cultured in HTF or G5 culture medium. We also did not observe any differences in the variance in methylation per amplicon between the two culture medium groups. A separate surrogate variable analysis also demonstrated that the IVF culture medium was not associated with the DNA methylation status of these DMRs. The mean methylation level and variance per CpG was equal between HTF and G5 placenta. Additional comparison of DNA methylation status of IVF placenta samples with naturally conceived placenta samples revealed no statistically significant differences in mean amplicon and CpG methylation between G5, HTF and naturally conceived placenta. However, the number of placenta samples exhibiting outlier methylation levels was higher in IVF placenta compared to naturally conceived ($p<0.00001$). Also, we were able to identify 37 CpG sites that uniquely displayed outlier methylation in G5 placentas and 32 CpG sites that uniquely displayed outlier methylation in HTF. In 8/37 (G5) and 4/32 (HTF) unique outliers CpGs, a medium specific unique outlier could be directly correlated to outlier methylation of the entire amplicon.
Limitations, reasons for caution: Due to practical reasons, not all placentas were collected during the trial and we collected the placentas from natural conceptions from a different cohort, potentially creating bias. We limited ourselves to 34 imprinted DMRs and we studied only the placenta tissue and no other embryo derived tissues.
Wider implications of the findings: It has often been postulated that imprinting mediates the effects of embryo culture conditions on pregnancy, birth and child development. We did not detect any statistically significant effects of embryo culture conditions on methylation status of imprinted genes in the placenta, therefore other unexplored mechanisms may underlie these effects.
Trial registration number: Placental biopsies were obtained under Netherlands Trial Registry number 1979 and 1298.

O-304 Risk factors for ectopic pregnancy after in vitro fertilization treatment: a case-control study

V. Trindade[1], M.R. Hentschke[1], V.C. Dornelles[2], A.T.F. Kira[1], T. Colombo[1], B. Cunegatto[3], L. Okada[3], A. Petracco[4], J.D.R. Michelon[5], B.E.P. Da Costa[2], M. Badalotti[4]

[1]Fertilitat - Center for Reproductive Medicine, Research, Porto Alegre, Brazil ;
[2]Pontifical Catholic University of Rio Grande do Sul, School of Medicine, Porto Alegre, Brazil ;
[3]Fertilitat - Center for Reproductive Medicine, Laboratory, Porto Alegre, Brazil ;
[4]Fertilitat - Center for Reproductive Medicine, Director, Porto Alegre, Brazil ;
[5]Fertilitat - Center for Reproductive Medicine, Medical Team, Porto Alegre, Brazil

Study question: Is it possible to identify risk factors for ectopic pregnancy after *in vitro* fertilization?
Summary answer: Tubal factor, previous miscarriage, D3 embryo transfer, two or more embryos transferred per cycle and oocyte recipient cycles were associate with ectopic pregnancy after IVF.
What is known already: Ectopic pregnancy is an obstetrical disease that is potentially associated with maternal death at first trimester. It is one of the well-known complications following IVF with embryo transfer (ET), as its incidence can reach 0.9-11% compared to 1–2% in spontaneous pregnancies. The etiology of a higher EP rate in an IVF-ET cycle remains unclear and the strategies to decrease its rates are limited.Thus, it is important to identify accurate risk factors for EP, as it represents not only a life-threatening event but also a lost opportunity for couples who sought assisted reproduction treatments.
Study design, size, duration: Retrospective case-control study performed at an assisted reproduction clinic in southern Brazil. To select the control group, a ratio of 1:4 was applied and collected from 4666 cycles that resulted in a clinical pregnancy after IVF-ET. In the end, 499 patients who underwent IVF cycle and evolved to clical pregnancy were included. The data refers to a period from 2001- 2019 and were collected from electronic records.
Participants/materials, setting, methods: The 499 patients were separated into groups: Group 1, who presented EP diagnose (n=90) and Group 2, intrauterine pregnancies (IUP) (n=409). Data were presented as mean ± standard deviation or percentage. Student t test, Mann–Whitney U-test, and Chi-square test or Fisher's exact test were applied. Multiple logistic regression analysis was performed to assess risk factors for EP. Statistical significance was defined as $p < 0.05$.
Main results and the role of chance: When compared Group 1 with Group 2 the following results were observed: Tubal factor infertility (35.6% *vs*. 21.1%, p=0.005) (OR 2.0 [1.2-3.4], p=0.004); History of previous miscarriage (15.1% *vs*.7.1%, p<0.05) (OR 2.0 [1.02–4.29], p=0.044); number of transfers in D3 embryo (69.2% vs. 54.0 p=0.028)(OR 1.9 [1.08 - 3.33], p=0.025); two or more embryos transferred (OR 2.5 [1.12–5.70], p=0.025). Even though we excluded oocyte recipient patients (ORP) from the analyzes above, we found a difference in ORP rate when comparing Group1 and group 2 (9.4% (10/106) vs. 3.0 % (13/434), p=0.007), (OR 3.3 [1.41 - 7.98] p = 0.005). No significant differences were observed in the analysis of female and male ages, body mass index, number of mature oocytes, sperm concentration, follicle-stimulating hormone,antral follicle count, male factor, ovulation factor, previous EP, previous pelvic surgery, endometrial thickness, embryo classification, protocol with agonist or antagonist or fresh versus frozen ET.
Limitations, reasons for caution: Retrospective study with limited number of patients. In addition, even though results show that oocyte recipient patients seems more likely to have an ectopic pregnancy, this data should be carefully interpreted given sample size.
Wider implications of the findings: Findings demonstrated a higher risk for EP in the group of patients who had tubal factor and previous miscarriage history. D3 transfer and more than one embryo per transfer were also associated

with higher EP risk. In high risk patients, it is reasonable to advise blastocyst and single embrio transfer.
Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS
SESSION 73: ENDOMETRIOSIS AND ART
08 July 2020 Parallel 5 14:00 - 15:15

O-305 Ultra-long administration of GnRH-a before in vitro fertilization does not improve the clinical pregnancy rate in women with mild endometriosis. A prospective, randomized, controlled trial

A. Kaponis[1], E. Panagodimou[1], G. Hadjopoulos[2], M. Paschopoulos[2], I. Georgiou[2], V. Paraskevaidis[2], K. Zikopoulos[2], K. Tsiveriotis[1], F. Taniguchi[3], G. Adonakis[1], T. Harada[3]

[1]Patras University School of Medicine, Ostetrics & Gynecology, Patra, Greece ; [2]Ioannina University School of Medicine, Obstetrics & Gynecology, Ioannina, Greece ; [3]Tottori University Faculty of Medicine, Obstetric and Gynecology, Yonago, Japan

Study question: Is there any beneficial effect of GnRH-a administration, post-laparoscopically, in the fertility of women with mild endometriosis who underwent in vitro fertilization (IVF) procedures?
Summary answer: The clinical pregnancy rate in mild endometriosis women who received GnRH-a, post-laparoscopy, did not improve compared with women who did not receive this regime.
What is known already: GnRH-agonists have long been administered for the treatment of endometriosis, however, their use postoperatively, in women with mild endometriosis has been linked with controversial results. Several studies associated prolonged use of GnRH-a before IVF with a higher pregnancy rate, while others showed no statistically significant improvement. On the other hand, long-term pituitary desensitization regimes may lower ovarian response to gonadotropins, affecting the success of assisted reproduction procedures.
Study design, size, duration: This is a prospective, randomized, controlled trial. Six hundred infertile women were recruited during a 15-year interval (May 2004- December 2018). Four hundred of the afore-mentioned women diagnosed with mild endometriosis (ASRM stage I-II), while 200 from tubal factor infertility (group C). Patients with endometriosis were divided randomly in those who received GnRH-a, for three months before an IVF attempt (group A, n=200) and those who did not (group B, n=200).
Participants/materials, setting, methods: Women aged 29-38 years, were recruited from three tertiary University hospitals (Ioannina and Patras, Greece; Yonago, Japan). There were no demographic discrepancies between groups. Long protocol for ovulation induction was used. TNF-a, IL-1β, IL-6, IL-8, and IL-1ra levels were measured in the follicular fluid (FF) of all women. Fertilization Rate (FR), Implantation Rate (IR), quality of embryos, and clinical pregnancy rate (PR) were evaluated and compared between the different groups.
Main results and the role of chance: Women with endometriosis who received the long-acting GnRH-a (group A) presented significantly decreased concentrations of the FF's cytokines compared with those who did not receive similar treatment (group B). This applied to TNF-a, IL-1β, IL-6, and IL-8 ($p < 0.001$). In women with endometriosis, FR and PRs were lower compared to women with tubal infertility. A statistically significant difference was observed only in the FR between women of group A and women of group B (group A: 72.7, 95%CI: 70.50-74.90 vs. group B: 61.7, 95%CI: 59.20-64.20, $p<0.05$). Embryo quality did not improve after administration of GnRH-a. The percentage of grade 1 embryos was comparable between the three groups (group A: 24.5, 95%CI: 21.50-27.50; group B: 21.6, 95%CI: 18.80-24.40; and control group: 26, 95%CI: 22.70-29.30). IR did not differ significantly between women of the three groups (group A: 18.4%, 95%CI: 14.6-22.2%; group B: 17%, 95%CI: 13-21%; group C: 19.3, 95%CI 15.5-23.1). Women of group A presented higher PR than women of group B however, this difference was not statistically significant (OR: 1.16, 95%CI: 0.73, 1.84, p=0.52). The study population is homogenous, adequately powered and the risk of bias reduced due to random allocation of women to the intervention or control group.
Limitations, reasons for caution: The long period for completion of the trial is a limitation of the current study. Active endometriotic lesions were cauterized before any reproduction procedure. This treatment might hided any beneficial effect of GnRH-a administration. Data on miscarriages and live birth rates are not available.
Wider implications of the findings: This study is expected to guide future management of infertile women with mild endometriosis undergoing IVF-ET. According to our data, these women better proceed directly to IVF-ET, after laparoscopy, since the administration of GnRH-a did not improve their fertility.
Trial registration number: NCT1269125

O-306 Oocyte survival and clinical outcome is impaired in young endometriosis patients after fertility preservation (FP).

A. Cobo[1], J. Giles[2], S. Paolelli[2], A. Coello[1], B. Vallejo[1], J. Serrano[1], J.A. García-Velasco[3], J. Remohí[2]

[1]IVIRMA-Valencia, IVF Laboratory-Cryopreservation Unit, Valencia, Spain ; [2]IVIRMA-Valencia, Ob/Gyn, Valencia, Spain ; [3]IVIRMA-Madrid, Ob/Gyn, Madrid, Spain

Study question: Is oocyte survival and clinical outcome impaired in endometriosis patients after FP when compared to elective freezers?
Summary answer: Oocyte survival, implantation, pregnancy and cumulative live birth rates are impaired in young (≤35y) patients with endometriosis.
What is known already: FP should be considered in women with endometriosis due to the progressive reduction in the ovarian reserve. In spite of the high incidence of the disease and the increasing number of endometriosis-women who have oocytes vitrified for FP, very little is known about the efficacy of the strategy in these cases. Currently, there is evidence of successful outcomes achieved after oocyte vitrification for elective FP (EFP), although the age of the patient is one of the most limiting factors. In this study, we compare the results achieved by endometriosis-patients after FP to those achieve by EFP patients according to age.
Study design, size, duration: Retrospective study including 485 (≤35y: N=123 and >35y: N=518) women with endometriosis and 641 (≤35y: N=123 and >35y: N=518) EFP patients. All women included had their oocytes vitrified for FP and returned to use their oocytes to attempt pregnancy.
Participants/materials, setting, methods: The Cryotop method was used for oocyte vitrification. Within the endometriosis group, 97.7% of patients were diagnosed with stage III-IV endometriosis and 47.8% had ovarian surgery prior to FP. Oocyte survival, implantation rate and clinical outcome were compared between endometriosis and EFP groups according to age (≤35y vs <35y). Values are expressed as mean and 95% confidence intervals (CI). Chi-square tests were used to compare the categorical data. $P<0.05$ was considered as statistically significant.
Main results and the role of chance: Mean age of patients with endometriosis by the time of FP was 35.7 ± 3.7 and 37.6 ± 3.5 for EFP patients. Lower outcomes were achieved in the group of women with endometriosis aged ≤35y ($P<0.05$): oocyte survival was 85.1% (95%CI 83.8-86.5) vs. 91.4% (95%CI 89.6-93.2), implantation rate was 38.6% (95%CI 45.7-63.8) vs. 54.5% (95%CI 33.9-43.3), clinical pregnancy rate was 49.2% (95%CI 42.9-55.5) vs.65.9% (95%CI 57.9-73.9), ongoing pregnancy rate was 40.9% (95%CI 34.7-47.1) vs. 57.7% (95%CI 49.4-66), and CLBR was 61.9% (95%CI 56-67.8) vs. 68.8% (95%CI 59.4-78.2) respectively ($P<0.05$). In older patients (>35y) all the parameters were comparable: 80.8% (95%CI 78.9-82.6) vs. 82.1% (95%CI 80.9-83.3) for oocyte survival, 28.3% (95%CI 23.1-33.5) vs. 33.8% (95%CI 28.1-39.4) for implantation, 41.4% (95%CI 34.0-48.8) vs. 36.5% (95%CI 32.5-40.5) for clinical pregnancy, 29.6% (95%CI 22.7-36.5) vs. 27.7% (95%CI 23.9-31.5) ongoing pregnancy and 28.4% (95%CI 19.7-37.7) vs. 25.5% (95%CI 21.1-29.9) for CLBR in endometriosis vs EFP respectively (NS).
Limitations, reasons for caution: Retrospective nature of the study. The lower number of patients diagnosed as stages I-II makes it difficult to compare by the stage of the disease.
Wider implications of the findings: The lower oocyte survival and the impaired implantation and reproductive potential observed in young endometriosis patients confirms the negative impact of the disease in the ovarian reserve

and in the oocyte quality. FP should be counseled to endometriosis patients at young ages in order to increase their future pregnancy chance.
Trial registration number: Not applicable

O-307 ICSI treatment does not result in improved live birth rates compared to conventional IVF in couples with endometriosis: An analysis of 10,047 treatment cycles

H. Kamali[1], I. Gamaleldin[2], A. Kaura[3], P. Wilson[4], V. Akande[1]

[1]Bristol Centre for Reproductive Medicine, Clinical team, Bristol, United Kingdom ; [2]North Bristol NHS Trust, Women and Childrens Health, Bristol, United Kingdom ; [3]Imperial College NHS Healthcare Trust, Medical statistics, London, United Kingdom ; [4]Bristol Centre for Reproductive Medicine, Embryology, Bristol, United Kingdom

Study question: Is ICSI associated with improved reproductive outcomes over conventional IVF in couples with endometriosis related infertility (ERI) only?
Summary answer: Compared to conventional IVF, ICSI results in reduced fertilisation failure but is not associated with improved implantation, clinical pregnancy or live birth rates in ERI
What is known already: Endometriosis can affect up to 50% of women with infertility. As well as anatomical distortion, the condition can also negatively affect oocyte quality, embryogenesis and implantation through altered folliculogenesis, sperm phagocytosis, impaired fertilisation and embryotoxicity. ICSI, which is typically used for male factor infertility and known to result in improved fertilisation rates compared with conventional IVF, is increasingly being used in the cohort of patients with ERI to try and overcome these effects. However, there are no large-scale studies directly assessing the benefit of ICSI over conventional IVF with regards to reproductive outcomes in women with ERI.
Study design, size, duration: Anonymised data on all IVF and ICSI treatment cycles performed in the UK from years 1991 – 2016 were retrospectively obtained from the Human Fertility and Embryology Authority (HFEA). Women under 40 having their first treatment cycle using fresh embryos with a diagnosis of ERI only were included.
Participants/materials, setting, methods: Primary outcome was live birth rate. Secondary outcomes were fertilisation rate, implantation rate and clinical pregnancy rates. Outcomes were further stratified by age group (<35, 35-37, 38-39) and time period to account for improved LBR over time. Data were analysed using logistic regression and controlled for age, number of oocytes collected, and number of embryos transferred.
Main results and the role of chance: A total of 10,047 women had ERI of which 80.3% (8,075) had IVF compared with 19.7% (1,972) who underwent ICSI. The proportion of couples with ERI having ICSI increased from 6.3% in 1991-2000 to 23.7% in 2011-2016. Rates of total fertilisation failure (TFF) were lower with ICSI compared with IVF (3.7% versus 6%, p-value <0.001). However, in women with ERI, ICSI did not result in an improved live birth rate (OR 1.05, CI 0.94-1.17, p-value 0.42), clinical pregnancy rate (OR 1.06, CI 0.95-1.17, p-value 0.33) or miscarriage rate (OR 1.07, CI 0.82-1.39, p-value 0.62) compared with conventional IVF. The absence of improved reproductive outcomes with ICSI compared to conventional IVF in women with ERI was consistent across all age groups and different time periods.
Limitations, reasons for caution: As a retrospective study, our analysis depends on previously recorded data, therefore certain key variables (endometriosis stage, presence/absence of endometriomas, clinic specific protocols and duration of infertility) could not be accounted for. The reported cause of infertility is based on the treating clinician's classification and may be inconsistent.
Wider implications of the findings: In couples with ERI, ICSI results in reduced TFF compared with conventional IVF. However, this does not translate to an improvement in post fertilisation reproductive outcomes, including live birth rate. These findings do not support the use of ICSI over conventional IVF for treatment of couple with ERI.
Trial registration number: NA

O-308 A prospective randomized comparative study of laparoscopic ovarian cystectomy and ablation versus transvaginal cyst aspiration in infertile patients with Endometrioma undergoing IVF – ET treatment

S. Saha[1], H.H. Shirazee[1], M. Murmu[1]

[1]North Kolkata Infertility & IVF Centre, Reproductive Medicine, Kolkata, India

Study question: Is transvaginal endometriotic cyst aspiration better than laparoscopic ovarian cystectomy for preservation of ovarian reserve?
Summary answer: Better ovarian reserve in transvaginal cyst aspiration group achieve more number of oocytes and embryos resulting in significantly higher cumulative pregnancy rate.
What is known already: Laparoscopic cystectomy causes decreased ovarian reserve due to loss of normal ovarian tissue and thermal damage leading to poor reproductive outcome.
Study design, size, duration: In this Randomized, Control Trial over 2 years (July 2017 to June 2019), 104 infertile patients of 20-36 years of age with endometrioma were divided into- Group A and Group B comprising 52 patients in each group
Participants/materials, setting, methods: In this study Group A-52 patients had laparoscopic chocolate cystectomy followed by 4 weekly injection GnRH-a depot(3.75 mg) for 3months followed by IVF-ET treatment whereas Group B-52 patients received 4 weekly Injection GnRH-a depot(3.75 mg) for 3months followed by transvaginal chocolate cyst aspiration and underwent IVF-ET treatment . Patients in both groups were evaluated for ovarian reserve, no. of oocyte retrieved, fertilization rate, No. of embryo, implantation rate, pregnancy rate & outcome.
Main results and the role of chance: In Group A & Group B, mean no of baseline antral follicles and AMH were 2.1 ±0.9 Vs 4.8 ± 0.6 ($p< 0.05$) and 1.98 ± 0.8 Vs 3.1 ±1.2 ($p< 0.01$) respectively. Baseline FSH was significantly more in Gr A (9.1± 1.4 Vs 6.6±1.3, $p= 0.04$). The number of average oocyte retrieved was significantly more in Gr B (7.2±2.2 vs 4.8±2.8, $p<0.05$). Cumulative pregnancy rate per ovum pick up cycle is significantly more in Gr B (60.4% vs 45.1% , $p<0.05$).
Limitations, reasons for caution: As this study is of short duration with small number of patients so widespread multicentric study comprising large number of subjects is requirred to cone to a definitive conclusion.
Wider implications of the findings: Treatment cost and patient's morbidity is less in group B with better ovarian reserve leading to more number of oocytes retrieved and number of good quality embryo available for transfer. Cumulative pregnancy rate following single ovum pick up cycle is significantly more in transvaginal cyst aspiration group.
Trial registration number: Not applicable

O-309 Endometrial scratching in women undergoing their first In Vitro Fertilisation (IVF) cycle: results from the UK Multicentre Endometrial Scratch Randomised Controlled Trial

M. Metwally[1], R. Chatters[2], C. Pye[1], M. Dimairo[2], D. White[2], S. Walters[2], J. Cohen[3], T. Chater[2], K. Pemberton[2], T. Young[2], R. Lomas[1], E. Taylor[1], S. Laird[4], L. Mohiyiddeen[5], Y. Cheong[6]

[1]Sheffield teaching hospitals, Reproductive medicine, Sheffield, United Kingdom ; [2]The University of Sheffield, Clinical Trials Unit, Sheffield, United Kingdom ; [3]Hull York Medical School, Hull Health Trials Unit, Hull, United Kingdom ; [4]Sheffield Hallam University, Biosciences and Chemistry, Sheffield, United Kingdom ; [5]Manchester University Hospitals, Reproductive Medicine, Manchester, United Kingdom ; [6]The University of Southampton, Reproductive Medicine, Southampton, United Kingdom

Study question: Does endometrial scratching (ES) in the mid-luteal phase prior to first time IVF/ICSI increase the chances of achieving a clinical pregnancy and live birth?
Summary answer: Endometrial Scratch performed prior to the first cycle of IVF/ICSI does not increase the chances of achieving a pregnancy and live birth.
What is known already: Endometrial Scratch is currently being provided in some fertility units where women are having IVF/ICSI for the first time. The most recent 2019 systematic review shows poor evidence to support this practice. Current studies are single centre, of relatively small size and/or involve heterogeneous or unselected populations. Further evidence is therefore required from a large multi-centre randomised controlled trial looking at a homogenous population of only women undergoing first time treatment and expected to be good responders in order to minimise heterogeneity.
Study design, size, duration: A multicentre, pragmatic, open-label, individually randomised parallel-group trial recruited women at 16 fertility centres

across the UK from July 2016 to Oct 2019. We planned to recruit 1044 women (522 per arm) to preserve a power of 90% and 5% two-sided type I error assuming a 30% live birth rate in treatment as usual (TAU), a 10% absolute difference between TAU and Endometrial Scratch (ES) arms (more likely to change practice), and 5% dropout rate.

Participants/materials, setting, methods: Women aged ≥18 and ≤37 undergoing their first IVF cycle, BMI <35 kg/m^2 and expected to be good responders were eligible. ES was undertaken in the mid-luteal phase prior to IVF/ICSI. Women were randomised 1:1 to either ES or IVF/ICSI (TAU), using a web-based stratified block randomisation. The primary outcome was live birth (LBR); secondary outcomes included clinical pregnancy (CPR), implantation (IR), ectopic pregnancy (ER), miscarriage (MR), preterm delivery (PDR), stillbirth (SBR) rates and safety.

Main results and the role of chance: 1048 women were randomised TAU (n=525) and ES (n=523). Mean age and BMI (standard deviation) was 32.5(3.4) years and 24.5 (3.3) kg/m^2. Baseline characteristics were similar between arms. In the ES arm, 86.6% (453/523) received the ES procedure. IVF/ICSI was received by 94.1% (494/525) in the TAU and 95.0% (497/523) in the ES arm. For the primary outcome, LBR was 37.1% (195/525) in the TAU and 38.4% (201/523) in the ES (Unadjusted: *difference*, 1.3% [95%CI, -4.6% to 7.2%]; *relative risk*, 1.03 [95%CI, 0.89 to 1.21]; *odds ratio*, 1.06 [95%CI, 0.82 to 1.36]; P-value = 0.667).

For the secondary outcomes, CPR was 40.6% (213/525) in TAU and 42.4% (222/523) in ES arm (Unadjusted: *difference*, 1.9% [95%CI, -4.1% to 7.8%]; *relative risk*, 1.05 [95%CI, 0.91 to 1.21]; *odds ratio*, 1.08 [95%CI, 0.84 to 1.38]; P-value = 0.538). All the other secondary outcomes (IR, EPR, MR, MBR, PDR, and SBR) were all similar between arms.

Adverse events and serious adverse events in women were similar between arms. No deaths or neonatal deaths were reported. Only 1.2% (3/258) of born babies had severe congenital abnormalities reported in the TAU arm only.

Limitations, reasons for caution: The study did not include a sham procedure in the Treatment As Usual arm but this is unlikely to have influenced the study outcomes Furthermore, the study is applicable to the demographic and ethnic distribution of the population in the UK as no overseas centres were included.

Wider implications of the findings: Endometrial scratch is currently performed for women undergoing first time IVF/ICSI in some centres. This study provides conclusive evidence that Endometrial Scratch is not beneficial in this population and suggests that it is time to stop this practice.

Trial registration number: ISRCTN23800982

SELECTED ORAL COMMUNICATIONS
SESSION 74: OOCYTE AND EMBRYO EVALUATION

08 July 2020 Parallel 6 14:00 - 15:15

O-310 Optical coherence microscopy as a novel tool for quality assessment of mammalian oocytes and embryos

A. Ajduk[1], M. Fluks[1], A. Sobkowiak[1], S. Tamborski[2], M. Szkulmowski[2]

[1]University of Warsaw, Faculty of Biology, Warsaw, Poland ; [2]Nicolaus Copernicus University, Institute of Physics, Torun, Poland

Study question: We wished to examine whether optical coherence microscopy (OCM), a novel technique of label-free live-cell imaging, can be used in the oocyte/embryo quality assessment.

Summary answer: OCM allows for non-invasive imaging of the intracellular architecture of oocytes and embryos. These morphological data can be predictive of oocyte/embryo developmental potential.

What is known already: Fluorescence microscopy (including its confocal version) allows for detailed structural and dynamic studies of single cells, but requires fluorescent markers to visualize cellular architecture and may cause short- and long-term photo-damage. Bright-field microscopy, although non-invasive, does not provide detailed structural information. OCM is a promising alternative, as it does not require sample pre-processing or labeling and is capable of providing three-dimensional images of intracellular structures, such as nuclei (with chromatin conformation and nucleoli), metaphase spindles and network of endoplasmic reticulum cisterns and mitochondria. It is believed that these morphological parameters can be useful for oocyte/embryo quality evaluation in IVF protocols.

Study design, size, duration: We tested the OCM on mouse oocytes and embryos, focusing so far on immature (so-called germinal vesicle, GV) oocytes and compacted morulas. We analyzed over 400 GV oocytes and over 400 morulas in order to verify whether OCM scanning affects their developmental capabilities and whether morphological data obtained by OCM (chromatin conformation in GVs and number of nuclei in morulas) correlate with their developmental potential.

Participants/materials, setting, methods: GV oocytes were isolated from ovaries whereas morulas were obtained by in vitro fertilization of ovulated oocytes. OCM scans of GVs and morulas provided morphological information that was correlated with their developmental potential tested as the ability to mature and react to fertilization (for GVs) or to achieve a blastocyst stage with well-differentiated embryonic cell lineages (for morulas). We also tested whether scanned oocytes/embryos differ in their developmental capabilities from their non-scanned counterparts.

Main results and the role of chance: We showed that OCM allows for a precise assessment of chromatin conformation in mouse GV oocytes (surrounded by cumulus cells or denuded) and for the selection of oocytes with so-called SN (surrounded nucleoli) chromatin conformation that have higher developmental potential. GVs scanned by OCM matured in vitro with the same speed and efficiency as non-scanned control oocytes and developed similar Ca^{2+} response to a fertilizing spermatozoon, indicating that OCM scanning did not affect them negatively. We also showed that OCM provides data that allows for a precise nuclei (i.e. cell) count in compacted mouse embryos and that the number of nuclei (cells) at the compacted morula stage correlates with the embryo's ability to form high-quality blastocysts. Morulas scanned by OCM developed to the blastocyst stage with similar efficiency as their non-scanned counterparts, although the differentiation of the primitive endoderm (PE) cells was slightly delayed. The number of PE cells in scanned embryos was lower at day 4.0 than in non-scanned embryos, but the difference disappeared at day 5.0, suggesting that the developmental potential of the embryos was not significantly disturbed by the OCM.

Limitations, reasons for caution: We tested OCM only on mouse embryos and in the case of other species, additional tests are required. Moreover, further experiments testing the potential effect of OCM on the post-implantation embryo development are necessary (and currently in progress).

Wider implications of the findings: Our data indicate that OCM is a non-invasive imaging technique and that it provides morphological information about mammalian oocytes and embryos inaccessible for standard bright-field microscopy. Therefore, it can be an interesting alternative for imaging techniques used currently in assisted reproduction protocols.

Trial registration number: n/a

O-311 Development of an Advanced Quality Control Bioassay for Assisted Reproductive Technologies

K. Sakurai[1], A. Tran[1], V. Tovar[1], R. Newman[1], N. Ciray[1]

[1]Fujifilm Irvine Scientific, Research and Development, Santa Ana, U.S.A.

Study question: Could embryos expressing GFP-tagged POU5F1, a transcription factor critical for embryonic development during preimplantation development, be used to detect adverse conditions resulting during ART practices?

Summary answer: The *Pou5f1-GFP* embryos detected suboptimal conditions as early as 48hours of exposure and subsequently after 96 hours.

What is known already: Growing concerns about safety of ART on human gametes and embryos have been raised by the observation of compromised embryo development and the outcome of the treatment during ART laboratory procedures performed under suboptimal conditions. Incorporating evaluations of gene expression essential for preimplantation development in an assay for screening materials and conditions could benefit risk assessment. Zygotic *Pou5f1* gene expression, essential for mouse development, starts around the 4-cell stage and later restricted to ICM. Mouse embryos expressing GFP under control of *Pou5f1* regulators formed a relevant functional assay for testing ART products prior to clinical implementation (Gilbert et al., 2016).

Study design, size, duration: *Pou5f1-GFP* transgenic mouse lines expressing GFP-tagged POU5F1 were generated to utilize nuclear localization of POU5F1 and to detect adverse culture conditions and epigenetic defect during preimplantation. *Pou5f1-GFP* expression were also used to visualize blastomere nuclei for cell counting in live cells. *Pou5f1-GFP* embryos were cultured for 96hrs under optimal or suboptimal oil overlay to observe POU5F1-GFP expression at different stages of mouse embryo development (from 2PN to expanded/hatching blastocyst).
Participants/materials, setting, methods: *Pou5f1-GFP* one-cell embryos (fresh or frozen) were cultured to blastocysts under uninterrupted conditions up to 96 hours in Continuous Single Culture Medium-Complete (CSCM-C, FUJIFILM Irvine Scientific) with control or suboptimal oil overlay (5, 7.5, or 10% adulterated oil) and observed daily. B6 one-cell embryos typically used in the standard mouse embryo assay (MEA) were cultured in parallel. Embryo development was evaluated at 48 hours (% ≥8-cell) and 96 hours (% Blastocyst) quantitatively and qualitatively, respectively.
Main results and the role of chance: Transgenic mice expressing *Pou5f1-GFP* are viable and fertile, and successful germline transmission and temporally and spatially regulated gene expression were confirmed. Zygotic *Pou5f1-GFP* gene expression started around the 4-cell stage and peaked after culturing for 72hrs, consistent with earlier reports. The nuclear localization of POU5F1-GFP in mouse embryos enabled a quantitative approach to the assay. The *Pou5f1-GFP* embryos cultured with 5, 7.5, and 10 % suboptimal oil overlays showed a noticeable delay in development (at 48hrs and 96hrs compare to the control and MEA groups). Fresh and frozen *Pou5f1-GFP* embryos performed similarly in detecting suboptimal conditions.
Limitations, reasons for caution: As observed with other mouse traits, embryo yields are correlated to harvesting efficiency.
Wider implications of the findings: *Pou5f1-GFP* embryos can be used in the one-cell mouse embryo assay to examine the effect of culture conditions during preimplantation development. With the heightened sensitivity, the functional impact of materials and procedures on overall embryo development including embryo health and defects can be adroitly evaluated in live cells.
Trial registration number: not applicable

O-312 Follicular fluid levels of IL-10 are associated with oocyte fertilization and early embryo development.

L. Hartigan[1], L. Glover[2], H. Groarke[2], L. O'Shea[3], M. Wingfield[4]

[1]Merrion Fertility Clinic and National Maternity Hospital- Dublin- Ireland, Merrion Fertility Clinic and National Maternity Hospital- Dublin- Ireland, Dublin, Ireland ; [2]Merrion Fertility Clinic, Merrion Fertility Clinic, Dublin, Ireland ; [3]University College Dublin, School of Agriculture and Food Science, Dublin, Ireland ; [4]Merrion Fertility Clinic and National Maternity Hospital, Merrion Fertility Clinic and National Maternity Hospital, Dublin, Ireland

Study question: Do follicular fluid cytokine profiles predict the developmental potential of an oocyte in ART?
Summary answer: Follicular fluid IL-10 levels were higher in follicles where oocytes failed to fertilize or develop to blastocyst than oocytes that developed to Day 5 blastocysts.
What is known already: Embryo quality is greatly influenced by the oocyte and follicular microenvironment from which it originates. Follicular fluid contains a range of factors that play important signaling roles, including steroid hormones, metabolites, growth factors and cytokines. Inflammatory cytokines mediate functional and structural changes associated with follicle growth and early oocyte maturation. In previous work, our group identified follicular fluid AMH and progesterone levels as predictors of oocyte and embryo quality and developmental capacity. In this study, we adopted a similar approach to further investigate follicular fluid cytokines and their association with oocyte development.
Study design, size, duration: This was a prospective study of women undergoing IVF/ICSI treatment in our fertility clinic.Women with diagnoses of PCOS or endometriosis were excluded (n=30).Follicular fluid (FF) was collected from the first follicle aspirated during oocyte retrieval.The corresponding oocyte was individually cultured to track its outcome.FF was assigned one of 3 groups: Group 1: oocyte failed to fertilise (n=14); Group 2:oocyte fertilized but failed to reach blastocyst (n=29);Group 3:oocyte developed into a Day 5 blastocyst (n=29).
Participants/materials, setting, methods: Study participants were similar with regard to age, BMI, parity, AMH levels, antral follicle count and number of oocytes collected. FF cytokine protein levels were assayed using a commercially available multiplex array (Cytokine 30-Plex Human Panel, ThermoFisherScientific) on the Luminex multiplex assay platform (Conway Institute, University College Dublin). Data were analyzed using GraphPad Prism; cytokine levels were compared using Kruskal–Wallis with Dunn's multiple comparison test and Bonferroni correction ($p<0.05$ considered significant).
Main results and the role of chance: FF levels of the inflammatory cytokines TNFa ($p=0.037$), IL-6 ($p=0.031$), IL-5 ($p=0.021$) and IL-10 ($p=0.002$) were higher in follicles where the oocyte failed to develop to blastocyst (Group 1 and 2) than those where the oocyte developed to Day 5 blastocyst (Group 3). Following Bonferroni correction to control for multiplex analysis (18 cytokines), only differences in IL-10 levels retained statistical significance ($P<0.05$). Area-under-the-curve (AUC) of IL-10 as a predictor of oocyte development to a Day 5 blastocyst in this patient cohort was found to be 0.73 (95% CI 0.618-0.85; $p<0.001$). Nineteen FF samples had IL-10 levels <9pg/ml, 14 of which were from oocytes that successfully developed to blastocyst stage. IL-10 threshold levels <9pg/ml (sensitivity 48%, specificity 88%, prevalence rate 40% in this cohort) correspond to a positive predictive value (PPV) of 73.4% with 26.6% chance of a false positive test result, a negative predictive value (NPV) of 72.1% with 27.9% chance of a false negative result and an odds ratio (OR) of 7.093 (95% CI 1.57-4.32; $p<0.001$).
Limitations, reasons for caution: The study cohort was limited in size, and clinical pregnancy rates and live birth outcomes are not yet available.
Wider implications of the findings: A robust, non-invasive biomarker of oocyte competence would have a major impact on the prediction of ART success, including likely success of oocyte vitrification for an individual woman. This study shows that FF IL-10 may help predict the ability of an oocyte to fertilise and to reach blastocyst stage.
Trial registration number: not applicable

O-313 Evaluation of fragmented embryos – what is the best way to predict its implantation potential?

S. Lahav-Baratz[1], I. Blais[1], M. Koifman[1], M. Dirnfeld[1,2]

[1]Division of Reproductive Endocrinology and IVF Lady Davis Carmel Medical Center- Haifa- Israel, Department of OB/GYN, Haifa, Israel ; [2]Technion – Israel Institute of Technology- Haifa- Israel, Ruth and Bruch Rappaport Faculty of Medicine-, Haifa, Israel

Study question: Can we predict the potential of fragmented embryos to implant, by using the general and our "in-house" model for embryo selection in a time-lapse incubator?
Summary answer: The algorithms developed for embryos evaluation by morphokinetics, significantly increase the accuracy of a single embryo selection in our laboratory, including embryos with fragmentation.
What is known already: Fragmentation in IVF embryos has been a marker for embryo deselection. Extensive fragmentation may be associated with reduced blastocyst formation and with increased incidence of chromosomal abnormalities. However, some of those embryos may yield pregnancy and delivery of a healthy child. The cut-off of fragmentation rate which still enables achievement of pregnancy is not defined clearly. Using models, based on time-lapse technology, (general models, and lab-specific), may contribute to the selection of embryos with fragments.
Study design, size, duration: In this retrospective study, 4210 embryos which were incubated to the blastocyst stage in the EmbryoScope between 2013 and 2019 were analyzed. Three hundred seventy nine embryos, which had more than 5% fragmentation, were included in the study. Embryos were selected for transfer or freezing versus de-selection, based primarily on the general model for day 5 provided by Vitrolife and then re-examined by our lab-specific algorithms.
Participants/materials, setting, methods: Embryo fragmentations were measured using EmbryoScope tools by a senior embryologist. Percentage of fragmentation was documented twice for every embryo: at the first cell division and in their maximum volume. The patterns of fragments accumulation during embryos development were followed. Data was analyzed using statistical methods for fragmentation with regards to patient's

age, insemination method, blastocyst formation, embryos transfer or freezing, known implantation data (KID) of the embryos, clinical pregnancy and live birth rates.

Main results and the role of chance: Up to 32% fragmentation the specific model score and fragmentation percent was found to be an independent variables. Embryos with up to 20 ± 12% fragmentation still had high score according to the first division time and usually transferred or cryopreserved. Significant difference in the fragmentation rate was found between embryos which reached the blastocyst stage and embryos which failed: 15± 11% and 36± 20% respectively ($p<0.0001$).

Fragmentation appeared usually at the first division and worsening was observed during the 3th or 4th divisions.

While no difference was found in fragmentation between embryos of standard IVF or ICSI, age had a significant negative effect on fragmentation ($p< 0.0001$). In this population of fragmented embryos, 33 of 379 embryos resulted in a delivery of a healthy child, 104 were negative and for 242, information is not available mainly due to freezing without thawing.

In 92.8% of the patients more than 1 embryo was with fragments.

In 64% of the patients fragmented embryos found in more than one cycle. All positive KID (known implantation data) had maximum of 30% fragmentation except one embryo with 43% fragmentation that was implanted and a healthy baby was born.

Limitations, reasons for caution: Although fragmentation was analyzed meticulously by experienced embryologists, measurement may be less accurate during late divisions. Prospective randomized studies are required to confirm these findings.

Wider implications of the findings: We suggest that fragmented embryos should be examined primarily according to their division timing. A specific model can contribute to a better selection. Although rare, even embryos with as much as 40% fragmentations and appropriate division timing may develop into a blastocyst which implant and results in a live birth.

Trial registration number: 0010-19-CMC

O-314 Assessment of the predictive value of mitochondrial DNA as a biomarker of embryo viability

M. Galain[2], M. Fabbro[2], S. Menazzi[2], R. Anria[1], J. Ponte[2], F. Nodar[3], S. Papier[1], C. Fernandez[2]

[1]Cegyr, Clinical, Buenos Aires, Argentina ;
[2]Cegyr, Genetics, Buenos Aires, Argentina ;
[3]Cegyr, Embryology, Buenos Aires, Argentina

Study question: Is the mitochondrial DNA (mtDNA) content related to female age, embryo morphology, ploidy, blastocyst implantation rate, pregnancy rate, and live birth rate?

Summary answer: There was a significant difference in mtDNA levels according to embryo ploidy and maternal age, but no difference regarding implantation, pregnancy and live birth outcomes.

What is known already: The mtDNA content of trophectoderm cells is related to the energy supply of the blastocyst, which could affect its implantation ability. It has been proposed that the quantity of mtDNA in trophectoderm (TE) biopsy cells can serve as a new biomarker of embryo viability. This information could help to maximize the success rate of single embryo transfers (SET) in ART by selecting embryos with the highest potential to achieve a live birth. However, the application of a score based on mtDNA is controversial and highly discussed.

Study design, size, duration: A cohort of 1528 embryos from 349 couples undergoing preimplantation genetic testing for aneuploidy between 2016 and 2019 was retrospectively included. Mosaic embryos (between 20%-80%) were excluded from this analysis. Blastocyst quality was established according to Gardner grading system and grouped in: high (3-6AA and 4-6AB), mid (any BB, 1-3AB and 1-2AA) and low (any AC, CA, BC, CB or CC) quality.

Participants/materials, setting, methods: The embryo ploidy and the mtDNA content were determined using NGS and bioinformatics algorithms. The mtDNA value was mathematically corrected according to embryo aneuploidy and gender. βhCG > 15 IU was considered to be positive for biochemical pregnancy. Ongoing pregnancy was defined as having a visible gestational sac with a fetal heartbeat at 8 weeks after SET. For the statistical analysis T-test, Kruskal-Wallis and Pearson correlation were applied. A p-value< 0.05 was considered statistically significant.

Main results and the role of chance: The average maternal age was 33.6 (21-46 years). From the 1528 blastocysts assessed, 624 were euploid (40,8%), 377 aneuploid (24,7%) and 527 mosaic embryos (34,5%). mtDNA was significantly different between euploid and aneuploid embryos ($p=0.0000038$) and between embryos from younger (≤37 years, n=592) and older women (>38 years, n=409) ($p=0.0002$). A positive correlation of mtDNA with maternal age was observed, but only in the group of older women the aneuploid blastocysts contained higher mtDNA vs. euploid embryos ($p=0.003$). There was no significant difference in mtDNA between high, mid and low quality euploid embryos and between the different grades of TE (A, B and C). However, a difference was found between aneuploid and euploid embryos in the mid and low quality categories. Regarding the pregnancy outcome, 264 euploid embryos were transferred, resulting in 112 ongoing pregnancies (42%) and a 61% live birth rate (n=69). There was no difference in mtDNA content between the positive βhCG vs negative βhCG, the ongoing pregnancies vs the non-pregnant group and the live birth vs abortions.

Limitations, reasons for caution: The study is limited by its retrospective nature. A higher sample size or a prospective randomized design in future studies would corroborate the current findings. Caution should be taken while comparing these results to other reported studies since the same quantification methodology is not always used.

Wider implications of the findings: This study contributes evidence that mtDNA quantification in euploid embryos has no clinical impact on pregnancy outcome. mtDNA score adds no value when selecting which embryo to transfer. It is unlikely that mtDNA quantification alone will be able to solve the complex scenery of embryo reproductive competence.

Trial registration number: not applicable

human reproduction

ESHRE 2020 / Poster Viewing

POSTER VIEWING SESSION
ANDROLOGY

P-001 Effect of sperm selection method by cumulus oophorus complexes and conventional sperm preparation method on sperm quality and DNA fragmentation for assisted reproduction techonology

W. Naknam[1], L. Salang[1], J.S. Sothornwit-[1], S. Amnatbuddee[1], K. Seejorn[1], T. Pongsritasana[1], S. Sookkasame[1]

[1]Department of obstetrics and gynecology Khon Khane university, obstetrics and gynecology, Khon Khane, Thailand

Study question: Are the effectiveness of sperm quality and DNA fragmentation different between sperm selection method by cumulus oophorus complexes and conventional sperm preparation method ?
Summary answer: Spermatozoa selected using COCs were likely to be effective in terms of sperm quality and DNA fragmentation.
What is known already: Spermatozoa are commonly selected for intracytoplasmic sperm injection (ICSI) visually under an optical magnification microscope based on motility and morphology. However, this method does not reveal the genomic integrity of the spermatozoa.Cumulus oophorus complexes (COCs) encircle the oocyte and contains cumulus cells and extracellular matrix (ECM). ECM is a main component in hyaluronic acid (HA), which is produced by COCs after an LH surge.The head of mature spermatozoa have a hyaluronan specific receptor that bind with hyaluronan. During natural fertilization, only one healthy spermatozoon can passthrough the COCs and zona pellucida and penetrate into the ooplasm for fertilization.
Study design, size, duration: This was a prospective experimental study. Thirty normal semen samples under the World Health Organization (WHO)'s 2010 eligibility criteria were collected and processed using conventional sperm preparation methods from June to October 2018.
Participants/materials, setting, methods: The prepared sperm were divided into two groups. Spermatozoa in the study group were selected based on their ability to penetrate a layer of COCs via COCs selected model. In the control group, spermatozoa were kept in culture medium under similar conditions. The selected-sperm were evaluated based on sperm quality and DNA fragmentation.
Main results and the role of chance:
Thirty normal semen samples were recruited. Spermatozoa that were able to passthrough the COCs had significantly higher sperm motility parameters than the control group (curvilinear velocity [VCL; 143.5 vs 122.2; $P < 0.01$], average path velocity [VAP; 83.6 vs 69.3; $P < 0.01$], straight-line velocity [VSL; 67.95 vs 60.45; $P < 0.01$]). The percentage of normal spermatozoa morphology in the COCs group was significantly higher than in the control group (21.70 % vs 18.76%). In addition, there was significantly less DNA fragmentation in the COCs group than in the control group (18.83 vs 10.83).
Limitations, reasons for caution: The limitation of this study, we did not evaluate other sperm functions, such as sperm capacitation, acrosome reaction, and spermatozoa-zona binding. However, these processes are unnecessary in ICSI because the ICSI usually bypass these processes.
Wider implications of the findings: The spermatozoa that were able to passthough COCs had higher sperm motility parameters, higher rates of normal morphology, and lower DNA fragmentation. It may be advantageous to implement this process during ICSI.This study showed that implementing a sperm selection method using an COCs model before ICSI may improve fertility outcomes.
Trial registration number: HE 611250

P-002 Sperm retrieval surgery in male infertility cancer survivors can result in live births.

H. Hibi[1], S. Miho[1], S. Megumi[2], F. Noritaka[2], A. Yoshimasa[2]

[1]Kyoritsu General Hospital, Urology, Nagoya, Japan ; 2Asada Ladies Clinic, Gyenecology, Nagoya, Japan

Study question: To determine the viability of sperm retrieval surgery in male infertility cancer survivors to restore the possibility of biological fatherhood.
Summary answer: Successful sperm retrieval was achieved in 40% cancer survivors, and 24% survivors became a biological fatherhood.
What is known already: Sperm cryopreservation can be offered to men with cancer prior to undergoing gonadotoxic treatments such as chemotherapy. However, cryopreservation of sperm prior to treatments is rarely done, whilst after treatment, it is severely compromised due to azoospermia or ejaculatory dysfunction.
Study design, size, duration: To determine the viability of sperm retrieval surgery in male infertility cancer survivors to restore the possibility of biological fatherhood. Data from 1,525 male infertility patients who consulted in our clinic between 2009 and 2018 were analysed. From this database, a retrospective analysis was done to identify patients with a history of past cancer treatment and who underwent surgical sperm retrieval due to azoospermia or ejaculatory dysfunction.
Participants/materials, setting, methods: Twenty-five patients were diagnosed as having azoospermia or ejaculatory dysfunction after cancer treatments. Type of cancers were testicular cancer (8 cases), hematologic cancer (6), colorectal cancer (3), osteosarcoma (2), kidney cancer (2), extragonadal cancer (2), gallbladder cancer (1), and rhabdomyosarcoma (1). Sperm retrieval surgery was performed using vasal sperm aspiration in 4 cases, epididymal sperm aspiration (2), testicular microdissection (18), and onco-testicular sperm extraction (1).
Retrieved sperm were cryopreserved for future ICSI treatment in our center.
Main results and the role of chance: Motile sperm were recovered in 10 cases (10/25; 40%), whereas immotile sperm in 2. No sperm was obtained in 13 (52%) cases. Six healthy deliveries were achieved with intracytoplasmic sperm injection following use of motile sperm in each of six cases (24%).
Limitations, reasons for caution: The size of our sample was limited due to the rarity of cancer cases in our clinic.
Wider implications of the findings: Successful sperm retrieval was achieved in 40% cancer survivors, and 24% survivors became a biological fatherhood. On the other hand, some patients abandoned infertility treatment due to the costs associate with sperm retrieval surgery and fertility treatment.
Trial registration number: Not applicable

P-003 Quantitative analysis of the sHLA-G protein in seminal plasma

A. Schallmoser[1], **N. Sänger**[1]

[1]*University Hospital Bonn, Department of reproductive medicine, Bonn, Germany*

Study question: To examine the paternal levels of the immunoregulatory sHLA-G protein in seminal plasma during IVF treatment and to investigate possible correlations with age and pregnancy outcome of the female partner.
Summary answer: High sHLA-G levels in seminal plasma of the male partner appear not to be required for pregnancy
What is known already: Recent studies revealed that maternal and embryonic contributions impact on HLA-G protein expression and might contribute to pregnancy success or failure.

Also in seminal fluid, different levels of sHLA-G have been detected.
Study design, size, duration: Retrospective study. 106 samples from male donors were obtained between March and October 2018.
Participants/materials, setting, methods: Analysis of 106 paternal samples during IVF treatment. Semen was separated from seminal plasma using a density gradient consisting of a 90% lower layer and a 45% upper layer. Enzyme linked immunosorbent assay (ELISA), SDS-PAGE and Western blot were performed to analyze and confirm the sHLA-G values in paternal seminal plasma and TESE samples.
Main results and the role of chance: We observed a significant negative correlation of male age with total sHLA-G amount (P 0.023, R −0.221) in seminal plasma. Testicular biopsy samples were analyzed and tested positively with sHLA-G ELISA. No significant difference of sHLA-G levels in seminal plasma and pregnancy outcome of the female partner was detected. We observed a statistically significant difference between female age [P = 0.010, Mann-Whitney U test] and pregnancy outcome. As expected, more ideal embryos and less non-ideal embryos were transferred in the pregnancy group. In univariate regression only female age significantly predicted pregnancy outcome (OR 0.896, 95% CI 0.81-0.99, P = 0.026), while male age showed a trend to significance (P = 0.075). Semen quality and sHLA-G were not significant. In multivariate logistic regression including all parameters, a trend for an independent predictive value of female age was still detected (OR 0.911, 95% CI 0.81-1.03, P = 0.123).
Limitations, reasons for caution: Study was retrospective and not randomized with a small sample size.
Wider implications of the findings: We observed a significant correlation of male age with total sHLAG amount. Our analysis showed a wide spread of sHLA-G protein levels in seminal plasma samples of male donors, supporting reports of previous studies. The determination of the origins of HLA-G in seminal fluid require further detailed studies.
Trial registration number: non applicable

P-004 Biomarkers of stress and male fertility

A. Steiner[1], **T. Spitzer**[2], **F. Sun**[3], **R.M. Coward**[4], **K. Hansen**[5], **S. Krawetz**[6], **N. Santoro**[7], **J. Trussell, C**[8]

[1]*Duke University, Obstetrics and Gynecology, Durham, U.S.A. ;*
[2]*Naval Medical Center Portsmouth, Reproductive Endocrinology & Infertility, Portsmouth- VA, U.S.A. ;*
[3]*Yale School of Public Health, Collaborative Center for Statistics in Science, New Haven- CT, U.S.A. ;*
[4]*UNC School of Medicine, Department of Urology, Chapel Hill- NC, U.S.A. ;*
[5]*University of Oklahoma College of Medicine, Department of Obstetrics and Gynecology, Oklahoma City- OK, U.S.A. ;*
[6]*Wayne State University School of Medicine, Department of Obstetrics and Gynecology, Detroit- MI, U.S.A. ;*
[7]*University of Colorado School of Medicine, Obstetrics and Gynecology, Aurora-Colorado, U.S.A. ;*
[8]*Upstate University Hospital, Urology, Syracuse- NY, U.S.A.*

Study question: Does stress, as measured by salivary α-amylase and cortisol, negatively impact male fertility, as measured by semen parameters and couple pregnancy and live birth rates?
Summary answer: Biomarkers of stress in males are not negatively associated with semen parameters or pregnancy and live-birth rates. Salivary cortisol is positively correlated with sperm count.
What is known already: Stress induces elevation in salivary cortisol and α-amylase. Prior studies have shown that women with high levels of salivary α-amylase have reduced fertility compared to women with normal values. A prior study of males suggested that higher levels of stress, as measured by a four-item questionnaire, was associated with poorer sperm quality. The impact of stress, as measured by salivary biomarkers, on male fertility is not known.
Study design, size, duration: 94 infertile men with sperm concentration ≤15M/ml, motility ≤ 40%, or normal morphology ≤4% enrolled in the Males, Antioxidants, and Infertility (MOXI) Trial, were included in this prospective cohort study. Couples were followed for up to 6 months. Couples attempted to conceive naturally during the first 3 months and with clomiphene citrate with intrauterine insemination in months 4 through 6 if not pregnant.
Participants/materials, setting, methods: Men provided a first morning salivary sample and semen sample at baseline. Salivary samples were analyzed for α-amylase and cortisol. Semen parameters and DNA fragmentation were measured in the semen samples. The associations between salivary α-amylase and cortisol and semen parameters or DNA fragmentation were assessed using linear regression models adjusting for male age. Salivary levels were dichotomized at the 80th percentile. Pregnancy and live birth rates in couples were compared using chi square testing.
Main results and the role of chance: Salivary levels of α-amylase were not associated with semen parameters or DNA fragmentation. Salivary levels of cortisol were not associated with DNA fragmentation or normal sperm morphology. For every 1 unit increase in salivary cortisol the total sperm count increased by 18.1 million (95% CI: 4.5-31.73), and the total motile sperm count increased by 12.6 million (95% CI: 4.5-20.6). Pregnancy rates did not differ for partners of males in the highest quintile of α-amylase (26% and 27%, p= 0.92) or cortisol (44% and 24%, p=0.11) compared to partners of males with lower values. Live birth rates did not significantly differ for partners of males in the highest quintile of α-amylase (21% and 20%, p=1.0) or cortisol (38% and 17%, p=0.07) compared to partners of males with lower values.
Limitations, reasons for caution: Men enrolled in the trial were infertile with abnormal semen parameters. These results are not generalizable to men without infertility.
Wider implications of the findings: Among infertile males, stress, as measured by salivary α-amylase and cortisol, does not appear to negatively impact male fertility, and higher levels of cortisol may increase sperm count. Future studies should investigate whether stress impacts semen parameters among men in the general population.
Trial registration number: Clinical Trials.gov NCT02421887

P-005 Alternations of sperm protein profiles to elucidate the mechanism of impaired spermatogenesis by cancer chemotherapy

T. Takeshima[1], **K. Shinnosuke**[1], **Y. Yasushi**[1]

[1]*Yokohama City University Medical enter, Department of Urology- Reproduction Center, Yokohama City- Kanagwa, Japan*

Study question: This study aims to analyze alterations in proteomic profiles and validate selected protein biomarkers of spermatozoa in men with history of undergoing cancer chemotherapy.
Summary answer: Cancer-associated protein was identified by liquid chromatography-mass spectrometer analysis and database searching. And the protein was validated by Western-blotting.
What is known already: As advanced cancer treatments have improved the prognosis of cancer survivors, these treatments such as chemotherapy have been known to cause harmful effect on fertile capacity. A few studies reported the alternation in proteins of spermatozoa between cancer patients and healthy donor and some proteins with different expression levels were identified between two groups.
Study design, size, duration: This research is a cross-sectional cell-line research of control versus treatment. A group of patients with a history of anticancer drug administration in cancer diagnosis was assigned as "cancer group"(n=3), and a fertile donor group was assigned as "control group"(n=3). Written informed consent was obtained from all patients and this study design was approved by institutional review board of Yokohama City University Medical Center.

Participants/materials, setting, methods: The original diseases of cancer group were non-Hodgkin malignant lymphoma(n=2) and soft tissue tumor(n=1). Measuring the total sperm count by CASA, they were adjusted to 6 million in all specimens, and protein concentration was adjusted by BCA assay. After trypsin digestion and desalting, the expressed proteins in spermatozoa were analyzed by LQ-MS/MS and database searching was performed in two groups. Validation was performed for the proteins with different expression levels by Western-blotting.
Main results and the role of chance: A total of 1,152 proteins and 5,268 peptides were identified by global proteomics in both groups. Sorted by max fold change of expressions (>5 folds) and ANOVA ($p<0.01$), 29 proteins were identified. Of these identified proteins, we focused on one protein, which is cancer-associated protein highly expressed in digestive tract and urinary tract. In sperm of patients with cancer, this protein was overexpressed 54.8-folds more than that of fertile donor. This protein co-works with other protein of T-cell proliferation factor. Regimens of cancer chemotherapy patients in cancer group received were ABVD and IFM, ADM, and VCM therapy, respectively.By the Western-blotting, expression of this protein was validated.
Limitations, reasons for caution: Limitations of this research was inability to compare the sperm before and after the administration of cancer chemotherapy, because spermatozoa were all cryopreserved for fertility preservation before treatment.
Wider implications of the findings: It was speculated that T cell proliferation was induced by interaction between these proteins after induction of cancer chemotherapy. Functional analysis of these proteins would provide clue to the mechanisms of impaired spermatogenesis after cancer chemotherapy.
Trial registration number: not applicable

P-006 Outcomes of Microsurgical Varicocelectomy in Men with Severe Oligospermia

F. Tenorio Lira Neto[1], A.D.F. Cavalcanti Filho[1], M.M.F. Santana[1], T.D. Silva[1], M.A.S. Freire[1], E.D.P. Miranda[2]

[1]Instituto de Medicina Integral Prof. Fernando Figueira, Department of Urology, Recife, Brazil ;
[2]Universidade Federal do Ceará, Departmen of Urology, Fortaleza, Brazil

Study question: Is microsurgical varicocelectomy useful in men with severe oligospermia?
Summary answer: Microsurgical varicocelectomy provides significant improvements in semen analysis parameters of men with severe oligospermia, allowing the couples to choose less invasive treatments.
What is known already: According to the literature, men with severe oligospermia (sperm concentration < 5 million sperm/mL) have a lower chance of semen analysis parameters (SA) improvement after microsurgical varicocelectomy (MV) when compared to men with mild or moderate oligospermia. However, recently, some authors have shown that MV can improve the SA parameters in some severe oligospermic men, potentially allowing for less invasive and costly assisted reproductive techniques.
Study design, size, duration: This is a retrospective cohort study in 38 consecutive men with severe oligospermia and clinically palpable varicocele who underwent MV by two male fertility specialists between June 2016 and September 2019.
Participants/materials, setting, methods: We included those men and who had at least one postoperative SA, and excluded those who were azoospermic. Baseline demographic and clinical characteristics, intraoperative findings, pre- and postoperative SA parameters, and SA improvement were evaluated. Semen analysis Improvement was defined as an increase of more than 10% in total progressive motile sperm count (TMSC).
Main results and the role of chance: Thirty-eight participants were included in the study. The mean age of the participants was 34 (±9) years, and bilateral varicoceles were present in 70% of the men. Regarding preoperative SA, the median sperm concentration was 1.4 (±3.1) x 10^6/mL, the median progressivel motility was 10 (±25) %, and the median TMSC was 0.2 (± 1.3) x 10^6. Intraoperative findings were, mean number of ligated veins of 11.9 on the left side and 9.3 on the right side; mean diameter of the largest vein of 3.5 mm on the left side and 2.6 mm on the right side. After a median follow-up of 120 (±60) days, 79% of the participants improved their TMSC, with a median improvement of 1.3 (±11.2) x 10^6. Furthermore, 24% of the participants achieved a TMSC greater than 5 million, giving them the possibility of intrauterine insemination, and 19% achieved a TMSC greater than 20 million.
Limitations, reasons for caution: The small number of participants and limited follow-up are the main limitations of this study
Wider implications of the findings: Although advanced techniques of assisted reproduction are commonly offered as a first line therapy for men with severe oligospermia caused by varicocele, this study demonstrates that microsurgical varicocelectomy can improve the SA parameters of such men, allowing these couples to choose less invasive treatments.
Trial registration number: not applicable

P-007 Effect of a high fat diet on the spermatogenesis in an obese mouse model

L. Ramos[1], D. Komninos[2], G. Van der Heijden[1], T. Van Herwaarden[3], A. Kiliaan[4], I. Arnoldussen[4]

[1]Radboudumc, Obst. & Gynaecology. div. Reproductive medicine intern 791, NIJMEGEN, The Netherlands ;
[2]Radboud University, Faculty of Biology, Nijmegen, The Netherlands ;
[3]Radboudumc, Medical Laboratory, Nijmegen, The Netherlands ; 4Radboudumc, Anatomy, Nijmegen, The Netherlands

Study question: Which changes in spermatogenesis are found in an obese/overweight Ldlr-/-.Leiden mouse model by using a high fat diet (HFD) ?
Summary answer: This study demonstrates that diet induced obesity leads to impaired testicular structure, probably due to the disturbance of the hypothalamic-pituitary-gonadal (HPG) axis.
What is known already: Obesity can disturb one or more of these regulatory factors, leading to impaired spermatogenesis and subsequently impaired fertility. To understand the effects of obesity on fertility at a cellular level, this study analysed the testes of low-density lipoprotein receptor knock-out (Ldlr-/-.Leiden) mice, which received a high fat diet and as such developed the typical symptoms related to obesity.
Study design, size, duration: Knock-out mice with a normal diet (chow diet, n=15- control group) or a high fat diet (HFD, n=15) were used. Hormonal blood levels, bodyweight of the animals at 36 weeks of age and analysis of adipose tissue (percentage of fat deposits) were measured. Differential diet started at 3 weeks of age until week 36 that the mice were sacrificed. Both testis (one fixed in Boiun and one snap frozen) were used for further analysis.
Participants/materials, setting, methods: For histological evaluation, Sertoli cells, spermatocytes and spermatids were counted, and morphological analyses of the testes were performed after haematoxylin-eosin stain. For analysis of sperm cell quality, a TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) staining was performed on testes slices to quantify the apoptotic cells. In addition, a chemical staining with chromomycin A3 (CMA3) was performed on spermatozoa obtained from the epididymis to examine the chromatin compaction of the sperm cell.
Main results and the role of chance: At 36-weeks of age, the control group mean weight was 37.82 ± 1.23 grams while the HFD fed mice was 49.56 ± 0.90 grams. The HFD fed mice had significantly more mesenteric and inguinal fat compared to the mice on a control diet (($p<0.001$). Plasma measurements of cholesterol, triglycerides, insulin, leptin, and testosteron were al significantly higher in HFD fed mice. No significant differences in the relative and absolute number of seminiferous tubules in the testis slides between both groups was observed. But, the HFD fed mice had a significantly increased number of loosely arranged cells in the seminiferous tubules, which a lower spermatids:spermatocytes ratio. The morphology of the seminiferous tubules in the testes was aberrant and the spermatogenic cycle of the seminiferous epithelium was disturbed in the testes of the HFD mice.

No significant increase of TUNEL-positive cells were observed between groups. CMA3 measurements (chromatin condensation) did not differ between groups (HFD mice= 3.5% ± 2.0; control=(2.4% ± 0.89) but it was increased compared to the wild type C57BL/6 mice (aprox.1% CMA3-positive cells).
Limitations, reasons for caution: In this study only mice with a LDL-receptor mutation were used. Our findings can be partly related to this mutation effect in the cells next to dietary feeding. The addition of a wild type mice with a normal of HFD diet will help to evaluate the effect of fat consumption.

Wider implications of the findings: These results are a substantial addition to the emerging evidence of the negative impact of obesity on male fertility, which is of great societal impact and will help with counselling and treatment of infertile couples.
Trial registration number: Not applicable

P-008 Evaluation of a fully automated semen quality analyzer (LensHooke™ X1) for home-based monitoring

A. Agarwal[1], M.K. Panner Selvam[1], C.T. Hsu[2]

[1]Cleveland Clinic, American Center for Reproductive Medicine, Cleveland, U.S.A. ; [2]The Taiwanese Association of Andrology, Male Infertility, Taipei City, Taiwan R.O.C.

Study question: Can LensHooke™ X1 serve as a home-based semen quality monitoring (SQM) device and offer accurate test results?
Summary answer: LensHooke™ X1 semen quality analyzer offers not only a user-friendly experience for at home use, but it also provides accurate and reproducible results.
What is known already: At-home semen analysis is a choice for men who are reluctant to visit the diagnostic laboratory for semen testing. Although home-based semen quality analyzers (HBSQA) are available in the market, the information regarding post-surgical SQM using HBSQA is not available in the literature. Most HBSQA tests for only one or a few parameters at a time and their results cannot replace laboratory analysis of semen specimen. LensHooke™ X1 semen quality analyzer, a fully automated portable device, measures more than 3 semen parameters and provides real-time results for user, making it a good candidate for semen quality monitoring.
Study design, size, duration:
(1) Accuracy evaluation: 20 participants performed sperm concentration and motility analysis using X1.
(2) Reproducibility, precision and usability evaluation: To simulate at home SQM, 45 participants (28 Male, 17 Female) measured the concentration of latex beads using X1 at home device. Tests were performed once a week for a period of 5 months (20 readings). Following the completion of the trial, 45 questionnaires were filled out to evaluate user experience.
Participants/materials, setting, methods:
(1) Semen analysis was conducted on 3 seminal aliquots from each leftover specimens. Sperm concentration and total motility were initially assessed by medical technologist using Makler™ counting chamber. Participants with no experience in lab work (n=10) and trained-lab professionals (n=10) subsequently performed semen analysis on these samples using X1 analyzer.
(2) To evaluate user-experience of X1 device, we recruited 45 participants of different age and educational background.
Main results and the role of chance:
(1) For the evaluation of accuracy in semen analysis, the results of semen testing by either the participants with no experience in lab work (n=10) or trained-professionals (n=10) presented high degree of accuracy in concentration (90 % vs 93 %) and total motility (90 % vs 90 %) compared to reference value. There were no significant differences between concentration and motility results between the two groups (p = 0.84; p = 0.94). This suggests that users can operate X1 and obtain accurate results without hands-on experience or additional training.
(2) Results from 45 participants showed a very high reproducibility (>98.6 %) for the measurement of latex beads concentration. Moreover, the questionnaire shows 96% (±5.73%, 95% CL) positive feedback on the overall impression of X1 and up to 98% (±4.09%, 95% CL) satisfaction rate with an average score of 3.32 out of 4 points. The current data indicates that LensHooke™ X1 is an accurate, reproducible and user-friendly device.
Limitations, reasons for caution: Use of latex beads instead of semen samples to evaluate the reproducibility and usability of LensHooke™ X1 may not mimic the results of a semen sample.
Wider implications of the findings: LensHooke™ X1 is an easy-to-use SQM device which appears to be well suited for long term monitoring of semen quality after reproductive surgery as well as for short term SQM. Future studies to evaluate the performance of LensHooke™ X1 for post-medical or surgical intervention specimens will be useful.
Trial registration number: CS17027

P-009 Semen exosomes upregulated trophocytes migration and invasion and its effects on trophocytes angiogenesis via the Notch1/NF-κB signaling pathway

Z. Xiao[1], P. Jiali[1]

[1]Wuhan University, Renmin Hospital, Wuhan, China

Study question: Does semen exosomes (Exos) affect trophocytes (Tros) biological behaviors and angiogenesis? And how does it work?
Summary answer: Semen Exos could promote Tros migration, invasion and angiogenesis via the Notch1/NF-κB signaling pathway, have no significantly effect on cell cycle and apoptosis.
What is known already: At present, researches on Tros mainly focus on the maternal, including hormone level, inflammatory factors, growth factors, etc. The study on the regulation of Tros biological behavior by semen may provide a new direction for elucidating maternal-fetal interface modeling.
Studies have shown that intrauterine infusion of SP can improve the implantation rate of IVF-ET. Semen Exos mainly exists in SP, it's considered to be an important medium for micro-environmental regulation of reproductive function. RNA-seq studies have shown semen Exos can regulate the expression of genes in endometrium. However, the effect of semen Exos on Tros has not been studied.
Study design, size, duration: 40 cases of normal quality semen (according to the WHO 5th edition standards) were collected to extract semen Exos at Renmin Hospital of Wuhan University. To investigate whether semen has an effect on Notch-1 expression in villus, a total of 12 villus (6 cases of pregnancy by IVF-ET as test group and 6 cases of pregnancy in natural way as the control) were collected for immunohistochemical staining.
Participants/materials, setting, methods: Exos were extracted and identified from 40 volunteers and Notch-1 were measured in villus from 6 patients conceived through IVF-ET and 6 patients conceived naturally. The human trophoblast cell line (HTR-8/SVneo cells) were co-cultured with 10ug/ml semen Exos for 48h. Then CCK-8 assay, transwell assay, flow cytometry, and angiogenesis assay were performed to determine cell proliferation, migration, invasion, apoptosis, cell cycle and tube formation. Q-PCR and western-blot analysis were performed to determine RNA and proteins.
Main results and the role of chance: Exos were successfully extracted from semen and identified by transmission electron microscopy, NTA analysis and western-blot analysis. After co-cultured with semen Exos, HTR-8/SVneo cells migration, invasion and tube formation were increased significantly ($P<0.05$, n=3). And the expression of HTR-8/SVneo cells biological function-related mRNA and protein, like Notch-1, NF-κB-VEGF, VEGFR-1, VEGFR-2 were significantly increased ($P<0.05$, N=3). While there were no significant changes in cell cycle and apoptosis ($P>0.05$, N=3). Mechanistic studies showed Notch-1 signaling pathway played an important role. The expression of Notch-1 in villus from patients conceived by IVF-ET was significantly lower than the control group. Furthermore, the semen Exos promoting effects could be significantly attenuated by Notch-1 inhibitor.
Limitations, reasons for caution: It remains unclear that which substance in semen Exos is the upstream signal of notch-1 signaling pathway.
Wider implications of the findings: The study of roles of semen Exos of maternal-fetal interface modifying is of great significance not only in exploring the origin of reproduction, but also in preventing and controlling pathological pregnancy, improving IVF-ET pregnancy outcome, developing new contraceptives, and improving the reproductive rate of rare animals.
Trial registration number: not applicable

P-010 Easy, efficient and safe sperm separation for IUI and IVF

A. Arav[1], Y. Barak[2], A. Komsky-Elbaz[3], D. Reches[4], D. Kalo[4], Z. Roth[4], Y. Natan[5], P. Patrizio[6]

[1]Fertilesafe Ltd., R&D, Nes-Ziona, Israel ; [2]Dr. Yona Barak Laboratory, Laboratory, Rosh Haayin Il, Israel ; [3]Department of Animal Science- Faculty of Agricultural- Food and Environmental Quality Sciences- the Hebrew University of Jerusalem-, Animal Science, Rechovot, Israel ; [4]Department of Animal Science- Faculty of Agricultural- Food and Environmental Quality Sciences- the Hebrew University of Jerusalem-, Animal Science, Rechovot, Israel ; [5]Fertilesafe, Animal Science, , Israel ; [6]Obstetrics- Gynecology & Reproductive Sciences- Yale Fertility Center & Fertility Preservation, In Vitro Fertilization, New Haven, U.S.A.

Study question: Is it possible to do easy and simplified sperm separation procedure using a new device named E. Sep.
Summary answer: E.Sep is an efficient and easy to use device allowing sperm separation from semen for IUI/IVF directly into a syringe by thermotaxis through micropores.
What is known already: Conventional separation of sperm includes various technologies such as two-step washing technique, density gradient centrifugation (DGC) and swim up. These techniques expose sperm to several noxious conditions such as: reactive oxygen species (ROS), contaminants present in the media or freezing solutions, air, pipetting, centrifugation steps, temperature shifts.
Study design, size, duration: Experimental research carried out in two different centers using animal and human sperm samples.
Participants/materials, setting, methods: Frozen/thawed bull semen (n=5) were mixed and processed (3 replicates) with 'swim-up' using three different techniques: regular swim-up (RSU); E.Sep in horizontal and E. Sep in vertical position. Spermatozoon mitochondrial membrane potential ($\Delta\Psi m$), ROS levels and acrosome membrane integrity were evaluated by flow cytometry, using specific kits (IMV Technologies). In humans, 3 fresh ejaculates were pooled and processed as 2 separated samples. Sperm concentration, motility and morphology (WHO 2010) was assessed before and after separation.
Main results and the role of chance: The animal experiments showed significantly higher $\Delta\Psi m$, reflected by the ratio of polarized/depolarized mitochondria, in E. Sep sperm groups compared to RSU (0.7 ± 0.30 vs. 0.3 ±0.01; P < 0.02). The $\Delta\Psi m$ tended to be higher in vertical E. Sep group (2.1± 0.45; P <0.1), compared to horizontal E. Sep. The oxidation level, reflected by the percentage of viable spermatozoa exhibiting ROS, was significantly lower in horizontal E. Sep (23.5% ± 5.83) and vertical E. Sep (4.8% ±1.24) compared to RSU (53.0% ± 2.00 ; P <0.008 and P<0.0007 , respectively). Acrosome integrity was significantly higher in horizontal E. Sep (13.1 ± 3.06%) and vertical E. Sep (29.4± 6.41) compared to RSU (3.2 ± 1.61%; P<0.02 and P<0.05, respectively).In the first human sperm pilot study (pool of 3 samples) we performed horizontal E. Sep. After 18 min at 37°C the number of motile sperm recovered in the syringe (volume 0.2mL) was $6x10^6$ with 12% normal morphology as compared to 3% normal morphology and 9.6 $x10^6$ motile sperm in the equivalent 0.2 mL unprocessed semen sample. In the second trial we recovered 4 $x10^6$ total motile sperm compared to 6 $x10^6$ in the unprocessed semen sample.
Limitations, reasons for caution: Further studies with higher number of patients and various sperm quality parameters, are currently planned.
Wider implications of the findings: This easy and safe method can be use for IUI, IVF and ICSI.
Trial registration number: not clinical trial

P-011 Examination of Spermatozoa CatSper membrane channel proteins by immunohistochemical method and investigation of their relationship with sperm parameters in normozoospermia

S. Yazıcı[1], T. Irez[2]

[1]T.C Biruni University, Histology and Embryology, İstanbul, Turkey ;
[2]Yeniyüzyıl University, Histology and Embryology, İstanbul, Turkey

Study question: Is the expression of Sperm CatSper protein related to sperm parameters?
Summary answer: It was determined that the concentration of the spermatozoa is related to the CatSper positive sperm ratio.No significant relations were detected with the Sperm motility.
What is known already: CatSper is a sperm-specific, voltage-dependent, Ca^{+2}-selective, pH-sensitive and positive-load channel, and also provides the passage of calcium ions. CatSper Cation Channels contribute directly to the mobility of the sperm, regulate acrosome reaction, and balance the intracellular pH. It is considered that the mutations and disorders in any subunit of the CatSper structure cause infertility. In a trial that was conducted in mice, the loss of α-subunits caused infertility. For this reason, it is an important cation channel for sperm hyperactivation and male fertility.
Study design, size, duration: In this prospective and controlled study, the 18-35 year-old normozoospermic male patients who applied to the Cebeci Hospital UYTE Center of Ankara University, Cebeci Hospital, between December 2018 and August 2019 were included. The Semen analysis was carried out according to WHO 2010 Criteria.
Participants/materials, setting, methods: Semen samples were taken from 50 patients who were diagnosed with normospermia. The sperms were prepared with the Swim-Up Method, and spread slides were prepared, and the İmmune Fluorescent Staining Method was used for CatSper protein. The Student's **t**-test and Pearson Correlation Test were used for statistical analyses.
Main results and the role of chance: The CatSper Protein was detected with the IHC Method in Principal Peace Region with the middle part. The relation of CatSper-positive sperms with sperm morphology and concentration was found to be statistically significant (p<0.05). No statistical significance was detected with sperm motility (p>0.05). The present study examined the effects of CatSper Channels on reproduction, and showed that it will open new ways to treat male infertility and for contraceptive purposes.
Limitations, reasons for caution: The inclusion of only 50 normospermic patients, and working with Swim Up samples in the study caused limitations in terms of the sampling.
Wider implications of the findings: The results of our study have the quality of pioneering future studies to investigate the relations of CatSper-expressing cells with pregnancy. It is aimed with a new study project that IVF results are investigated in terms of male infertility and unexplained infertility.
Trial registration number: None

P-012 Intra-Uterine Insemination (IUI): Is there an upper cut-off level of the Number of Motile Spermatozoa Inseminated (NMSI)? An analysis of 2.642 IUI cycles.

L. Delaroche[1], H. Caillou[2], F. Lamazou[1], E. Genauzeau[1], P. Meicler[1], P. Oger[1], C. Dupont[3], P. Humaidan[4]

[1]Ramsay Santé, Hôpital Privé de Parly 2- IVF Center, Le Chesnay, France ;
[2]Capionis, Capionis, Bordeaux, France ;
[3]Sorbonne Université, Saint Antoine Research center- INSERM équipe Lipodystrophies génétiques et acquises. Service de biologie de la reproduction-CECOS- AP-HP- Hôpital Tenon, Paris, France ;
[4]Aarhus University, The Fertility Clinic- Skive Regional Hospital Faculty of Health, Skive, Denmark

Study question: Does an upper cut-off level of the NMSI exist, above which the Live Birth Rate (LBR) is negatively affected?
Summary answer: No upper cut-off level for NMSI was found in IUI cycles. However, the LBR correlated positively with the NMSI until 30 million.
What is known already: Several authors tried to determine a lower threshold of post-washed motile sperm in IUI. However, to date no data have been published regarding a possible upper cut-off level of motile sperms, above which the LBR is negatively affected. Importantly, IVF laboratories are often used to diluting sperm preparations when the NMSI exceeds 10 million. The objective of this study was to determine the predictive value of the post-washing NMSI on the LBR.
Study design, size, duration: A total of 2.642 IUI cycles carried out from January 2010 to July 2018 with an NMSI >1 million were retrospectively analyzed. Patients under the age of 43 years were stimulated, using either clomifene citrate, FSH or combinations. Sperm was prepared by swim-up selection. IUI cycles using donor sperm or frozen-thawed sperm were excluded. The institutional review board approved the study and all patients gave written informed consent to the use of their data.
Participants/materials, setting, methods: A multivariate logistic regression model was used to assess the influence of NMSI of >10-20, >20-30 and >30 million on LBR compared to a NMSI of >1-10 million. In addition to NMSI, this model was adjusted for all clinically relevant factors, i.e. having demonstrated their influence on LBR as part of a preliminary selection step, using univariate logistic regression. The odds ratio (OR) and their 95% bilateral confidence interval from the multivariate model were calculated.
Main results and the role of chance: A total of 2.642 IUI cycles in 1.036 couples were included. Mean female age was 33.5 ± 4.4 years at the time of the first IUI. The mean NMSI per cycle was 9.0 million [1-97]. The LBR increased with the NMSI until 30 million without any maximum threshold (AUC = 0.5438). Using NMSI categories, the LBR per IUI cycle was 14.4%, 17.8%, 22.9% and 6.8% for NMSI between >1-10, >10-20, >20-30 and >30 million, respectively (p=0.003). Univariate analysis showed that the NMSI, female age, the number of mature follicles and the estradiol level on day of ovulation trigger, the cycle rank and the etiology individually influenced the LBR. Multivariate analysis,

adjusted for clinically relevant factors, showed that the LBR was 1.49 and 1.76 times higher when IUI was performed with a NMSI of >10-20 million (OR [95%CI] = 1.49 [1.10; 2.01]) and of >20-30 million (OR [95%CI] = 1.76 [1.07; 2.91]), respectively compared to IUI with an NMSI of >1-10 million (p=0.0119).

Limitations, reasons for caution: Although more than two thousand cycles were included and the main outcome was LBR, the design was retrospective and all cycles were performed in a single center.

Wider implications of the findings: The LBR after IUI can be optimized by inseminating a maximum of motile sperms. IUI preparations should not be diluted when more than 10 million motile sperms are obtained. It remains to be determined whether a dilution is necessary beyond 30 million. These preliminary results call for a prospective RCT.

Trial registration number: COS-RGDS-2019-09-004-DELAROCHE-L

P-013 Men's waist circumference in relation to infertility treatment outcomes among couples undergoing assisted reproductive technologies

H. Bian[1,2], L. Mínguez-Alarcón[3], I. Souter[4], J. Chavarro, E.[1,5,6]

[1]Harvard T.H. Chan School of Public Health, Department of Nutrition, Boston, U.S.A. ;
[2]Peking University School of Public Health, Institute of Reproductive and Child Health and Department of Epidemiology and Biostatistics, Beijing, China ;
[3]Harvard T.H. Chan School of Public Health, Department of Environmental Health, Boston, U.S.A. ;
[4]Massachusetts General Hospital Fertility Center and Harvard Medical School, Vincent Department of Obstetrics and Gynecology, Boston, U.S.A. ;
[5]Harvard T.H. Chan School of Public Health, Department of Epidemiology, Boston, U.S.A. ;
[6]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, U.S.A.

Study question: Is there an association between men's waist circumference and ART outcomes?

Summary answer: Men's waist circumference was inversely related to pregnancy and live birth rates among couples undergoing ART independently of men's and women's body mass index (BMI).

What is known already: Female adiposity, overall and central, has been related to worse ART outcomes. Likewise, men's obesity has been related to poor semen quality and may also have a negative effect on ART outcomes. Waist circumference as a measure of central obesity is also negatively associated with semen quality parameters in men of subfertile couples. Whether men's waist circumference impacts a couple's fertility independently of their and their partner's BMI is unknown.

Study design, size, duration: We utilized data from the Environment and Reproductive Health (EARTH) Study, a prospective cohort study, which recruited subfertile couples seeking evaluation and treatment at the Massachusetts General Hospital Fertility Center. Measurement of waist circumference was introduced to the study in October 2009. This analysis includes 179 couples undergoing ART utilizing their own gametes, with complete anthropometric information, recruited through January 2019.

Participants/materials, setting, methods: Men's and women's height, weight and waist circumference were measured on-site at baseline by trained personnel. Clinical treatment outcomes were extracted from the medical records. We analyzed the association of men's waist circumference and clinical outcome measures using cluster-weighted generalized estimating equation models to account for repeated treatment cycles of the same couple and adjust for men's age, BMI, height, race, education level, smoking status, and women's age, BMI, waist circumference and height.

Main results and the role of chance: Men's median (interquartile range, IQR) age, waist circumference, and BMI were 36.6 years (32.9-40.1), 95 cm (89-103) and 26.5 kg/m^2 (24.2-29.1), respectively. The correlation coefficient of men's waist circumference and BMI was 0.58 (P <0.0001). The correlation coefficients between men's and women's BMI and waist circumference were 0.29 and 0.40 (P <0.0001), respectively. Men's waist circumference was unrelated to fertilization rate overall and when IVF and ICSI cycles were separately examined. However, men's waist circumference was inversely related to clinical outcomes, including implantation, clinical pregnancy and live birth rates per initiated cycle. For each 5 cm increase in men's waist circumference, the odds (95% CI) of implantation, clinical pregnancy and live birth per initiated cycle decreased by 14% (2-24%), 12% (2-21%) and 9% (1-17%), respectively, after accounting for anthropometric and demographic characteristics of both partners. Results were comparable when waist circumference was modeled in categories using tertiles of the observed distribution, when using WHO suggested cutoffs for abdominal obesity, when restricting analyses to men with a normal BMI, and when restricting the analyses to fresh embryo transfer cycles.

Limitations, reasons for caution: As is the case of all observational studies, residual confounding cannot be ruled out. In addition, it is not known whether these findings are generalizable to couples attempting conception without medical assistance.

Wider implications of the findings: Men's abdominal adiposity, as measured by waist circumference, may adversely impact ART outcomes, even in the absence of obesity as defined by BMI cutoff values. These results suggest that central obesity may be an independent risk factor for male factor infertility.

Trial registration number: not applicable

P-014 Testicular sperm extraction (TESE) and genetic analysis feedback in a non-obstructive azoospermic man presenting a 46,XY/46,XX constitutional chimerism.

X. Ferraretto[1], J.F. Hermieu[2], M.A. Llabador[1], E. Amar[3], A. Hercent[4], J.P. Siffroi[4]

[1]AP-HP - Hôpital Bichat-Claude Bernard, Service de Biologie de la Reproduction, Paris, France ;
[2]AP-HP - Hôpital Bichat-Claude Bernard, Service d'Urologie, Paris, France ;
[3]Cabinet d'Andrologie Victor Hugo, Andrologie Urologie, Paris, France ;
[4]AP-HP – Hôpital d'Enfants Armand Trousseau, Département de Génétique Médicale, Paris, France

Study question: What is the sex ratio of spermatozoa incoming from spermatogenesis of a man presenting a 46,XY[70%]/46,XX[30%] constitutional chimerism?

Summary answer: Sex ratio of testicular spermatozoa analysed by fluorescent in situ hybridization (FISH) was 1:1, confirming that spermatogenesis is only conducted by 46,XY spermatogonia.

What is known already: Constitutional chimerism results from in utero fusion of two different zygotes in one single embryo. However, its prevalence is not well known, and only a few cases of 46,XY/46,XX chimerism have been reported to date. Moreover, no clinical case has been published on the exploration of spermatogenesis in 46,XY/46,XX chimerism by FISH analysis performed on spermatozoa after TESE.

Study design, size, duration: We studied a 35 years old patient with non-obstructive azoospermia. The patient was not issued from a twin pregnancy and had no history of heterologous stem cell transplantation. He presented a normal male phenotype but with a clinical bilateral testicular hypotrophy (left=4mL and right=8mL). Hormonal exploration results were: FSH=16.0UI/L, LH=10.5UI/L, Testosterone=3.68ng/mL and Inhibin B=18pg/mL. Genetic analyses performed on peripheral blood cells showed a 46,XY[70%]/46,XX[30%] chimeric karyotype, and no chromosome Y azoospermic factor (AZF) deletion.

Participants/materials, setting, methods: A bilateral TESE was performed by a senior urological surgeon. Sperm extraction was performed using swim-up and centrifugation. A few normal motile spermatozoa were retrieved on both sides and frozen. Remaining fresh testicular tissue was used for sperm analysis by FISH using direct hybridization probes coding for X (DXZ1) and Y (DYZ3) chromosomes and for chromosome 18 (D18Z1) as control of haploidy. FISH was performed separately on spermatozoa retrieved from right and left testicular tissue

Main results and the role of chance: On the right side, 96 nuclei were observed and results showed nearly identical rates of X and Y bearing spermatozoa: nuc ish(DXZ1x1,D18Z1x1)[45]/(DYZ3x1,D18Z1x1)[51]. Similar results were found on the left side after the analysis of 100 sperm nuclei: nuc ish(DXZ1x1,D18Z1x1)[49]/(DYZ3x1,D18Z1x1)[51].

These results highlight a 1:1 sex ratio on spermatozoa analysed from both testicular tissues, thus strongly suggesting that spermatogenesis was initiated from 46,XY spermatogonia. Performing FISH separately on both sides limited the risk of error and the role of chance.

Frozen testicular sperm was used in intracytoplasmic sperm injection (ICSI) and resulted in the birth of a healthy boy following the first embryo transfer.
Limitations, reasons for caution: Given that phenotypes are highly variable in this type of patient and are also related to the proportion of the two cellular contingents, our results in terms of presence of testicular spermatozoa and genetic analysis cannot be applied to all patients with 46,XX/46,XY chimerism.
Wider implications of the findings: This clinical case shows that it is possible to find usable sperm with a 1:1 sex ratio after TESE. These data can be used to inform future patients with this genetic abnormality about the safety and potential success of this procedure, without transmitting this genetic abnormality to their offspring.
Trial registration number: not applicable

P-015 A systematic review of at-home semen analysis technologies: A potential supplement to the fertility clinic?

F. Willendrup[1], E. Andersen[1], C. Panayotidis[2]
[1]ExSeed Health ApS, Denmark, Copenhagen, Denmark ;
[2]Gynaecology, Gynaecology, Isle of Wight, United Kingdom

Study question: The aim was to determine the use and validity of current at-home semen tests (HST), and their screening potential of male subfertility.
Summary answer: Most HST's are user friendly, accurate and effective diagnostic tools for primary and secondary care services.
What is known already: Semen analysis remain the main tool for diagnosis of male fertility status. Conventional spermograms use either automated or manual evaluation under a microscope. A basic predictive parameter is the Total Motile Sperm Count, which correlates well with pregnancy outcomes. HST provide an immediate result on basic semen parameters increasing the flexibility and easiness for the men. Although HST technologies are recently commercialised the general gynaecologists, practitioners and fertility specialists are not aware about their existence. The use of HST in primary and secondary care could improve the early correction of subfertility conditions and potentially improve pregnancy rates.
Study design, size, duration: The research protocol followed the published methodology for Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P), including their extension for Diagnostic Test Accuracy (PRISMA-DTA). The literature search included online databases with a predefined search strategy. Additional search was performed across online markets to find available tests not yet included in scientific reporting. All published material up to and including 12th of January 2020 was included.
Participants/materials, setting, methods: All available HST's were detected via research online, including any relevant applications in App Store (iOS) and Google Play (Android). All articles published on testing methods or devices used for analysing semen samples at-home or outside the conventional laboratory were included. Each technology and test were reviewed based on adapted predefined checklist from PRISMA-DTA.
Main results and the role of chance: 12 home sperm tests were identified and included in this review. Across the technologies, seven test methods were identified for the semen analysis, including manual microscope, automated microscope, microfluidics antibody reaction, paper-based antibody reaction, paper-based antibody reaction, microfluidic centrifugation, and mail-in tests. Some of them were smartphone enabled while others were paper-based. The accuracy of these tests was between 95-98 % and the "time until results" ranged between a few seconds to several days.

The majority of HST's give qualitative information on either the concentration or motile concentration. Most of the HST provide only the user with interpretation of the single sperm parameter result.

New high tech HST's include more in-depth analysis of several parameters. It is suggested by the authors that HST's can now aid further in improving patient experience and fertility awareness.
Limitations, reasons for caution: Material for some of the tests is unreliable, incomplete and subject to commercial imperatives. The study did not assess user experience and reproductive outcomes.
Wider implications of the findings: Our review shows that some HST are now measuring multiple sperm parameters, as well as reaching a high accuracy, making them valid tools for semen analysis. We propose a way for these technologies to overcome current clinical limitations, to improve patient care and ultimately treatment outcomes.
Trial registration number: Not applicable

P-016 Improvement of semen parameters by pre-conception care for male partners of infertility couples

A. Komiya[1,2], N. Uchida[3], A. Kuga[3], M. Ibayashi[3], K. Hiraoka[3], K. Kawai[3], T. Ichikawa[1]
[1]Chiba University Hospital, Urology, Chiba, Japan ;
[2]Kameda IVF clinic Makuhari, Urology, Chiba, Japan ;
[3]Kameda IVF clinic Makuhari, Reproductive Medicine, Chiba, Japan

Study question: This study was conducted to analyze whether lifestyle modification as a part of preconception care could improve semen parameters among the male partners of couples who visited a fertility clinic.
Summary answer: Lifestyle modification as a part of pre-conception care for male partners improved semen parameters without urologic intervention.
What is known already: Pre-conception care is the provision of biomedical, behavioral and social health interventions to women and couples before conception occurs. It aims at improving their health status, and reducing behaviors and individual and environmental factors that contribute to poor maternal and child health outcomes. Such care is also required for male partners of infertility couples. Recent Japan's National survey for male infertility revealed that 42.1% of male factor infertility was idiopathic, suggesting that at least some of these were caused by inappropriate lifestyle factors.
Study design, size, duration: This is a single-arm, single-center, retrospective observation study for a duration of one year after June in 2018 with 203 consecutive subjects.
Participants/materials, setting, methods: Non-azoospermic male partners of infertility couples were enrolled and asked about their lifestyles. Median age of male patients was 35 years old. These patients were promoted to modify their inappropriate lifestyles as pre-conception care before urologic evaluation. The lifestyles include factors such as smoking, chronic alcohol use, inappropriate underwear use, excessive body weight, length of abstinence and so on. Semen parameters was compared between before and after the promotion by student T or chi-square tests.
Main results and the role of chance: Forty-seven men (23.2%) were current smokers. 94 (46.3%) were chronic alcohol users. The lifestyles elevating scrotal temperature including fitted underwear and/or excessive time bathtub/sauna use were found in 150 (73.9%). Body mass index (BMI)=or>30 was found in 16 (7.9%). Blood tests revealed hypogonadism in 27 (13.3%), zinc deficiency in 30 (14.8%), abnormal lipid metabolism in 58 (28.6%), abnormal liver function in 42 (20.7%). Palpable varicoceles were found in 41 (20.2%). Erectile dysfunction was found in 80 (39.6%). Most of patients (200, 98.5%) had at least one factor. Then, changes in semen parameters were compared before and after the promotion of lifestyle modification. Median intervals of semen analyses were 28 days. Abstinence was decreased from 3.7 to 2.8 days in average (p=0.0135). Oligozoospermia was decreased from 99/203 cases (48.8%) to 77/197 (39.1%, p=0.0511). Asthenozoospermia was decreased from 160/203 (78.8%) to 91/196 (53.6%, p<0.0001). Oligoasthenozoospermia was decreased from 81/203 (39.9%) to 47/196 (23.7%, p<0.0001). Total motile sperm count was improved from 18.7±27.3 million to 33.1±44.6 million (mean±standard deviation, p<0.001). However, such improvement was not significant in those with palpable varicocele (4.7±25.0 million increase, p=0.2488), hypogonadism (22.3±58.4 million increase, p=0.0685), BMI=or>30 (33.1±69.2 million increase, p=0.0753), and zinc deficiency (8.3±34.2 million increase, p=0.1947).
Limitations, reasons for caution: This study was a single-arm, single-center, retrospective study with relatively small sample size. Results could be different if the study is conducted by double-arm, multi-center and prospective fashion in larger sample size.
Wider implications of the findings: Most of male partners of infertility couples had lifestyle and/or medical factors to decrease semen quality. Promotion of lifestyle modification should be attempted before and during urological/medical intervention. However, a short-term modification was not enough in those with varicocele, hypogonadism, zinc deficiency and excessive BMI.
Trial registration number: not applicable

P-017 A new small-molecule inhibitor of BRDT shows reversible male contraception effect in mice model

X. Li[1], S. Wu[1], J. Hou[1], M. Yin[2], W. Xu[3]

[1]Sichuan university, West China Second University Hospital, West China School of Medicine ;
[2]Central South University, Department of Dermatology- Huan Engineering Research Center of Skin Health and Disease- Hunan Key Laboratory of Skin Cancer and Psoriasis- Xiangya Hospital, Changsha, China ;
[3]Sichuan university, Joint Laboratory of Reproductive Medicine- SCU-CUHK- Key Laboratory of Birth Defects and Related Diseases of Women and Children- Ministry of Education- West China Second University Hospital, West China School of Medicine

Study question: To date, a reversible, reliable and oral medication to male contraceptive has not been successfully applied in the clinic.
Summary answer: We have discovered a dose-dependent small molecule Inhibitor of BRDT that can reversibly achieve male contraception through oral medication in animal experiments.
What is known already: Effective male contraceptives include condoms and vasectomy, but these methods are not ideal for all men. The bromodomain (BRD) and extra-C terminal domain (BET) protein family consists of four members (BRD2, BRD3, BRD4 and BRDT). These bind to acetyl lysine (KAc) residues on the tails of histones H3 and H4, and regulate chromatin structure and gene expression. The testis-specific BET member, BRDT, is essential for the normal progression of spermatogenesis and mutations in the Brdt gene result in complete male sterility in mice model. Therefore, promising method acts by blocking Brdt involved in the process of spermatogenesis for male contraception.
Study design, size, duration: We used 60 male mice divided into six groups (0mg/kg, 1mg/kg, 2mg/kg, 3mg/kg, 4mg/kg, 5mg/kg) treated daily from 6 to 14 weeks with NHWD-870, a potent BRDT inhibitor. After drug or vehicle treatment, mice were either sacrificed or mated to females while continuing to receive NHWD-870.
Participants/materials, setting, methods: C57BL/6 mice model, gene cloning, mouse contraceptive studies, hematoxylin-eosin staining, sperm counts and motility, sex hormone radioimmunoassay, immunohistochemistry, western blot, Immunofluorescence.
Main results and the role of chance: NHWD-870 was the most potent inhibitor of BRDT with a biochemical IC50 of 2.5 nM, 13.1nM, 2.3nM, 13.93nM on GC1, GC2, TM3 and TM4 cell lines proliferation. We tested spermatogenic effects of NHWD-870 administered to male mice. High dose groups (4mg/kg and 5mg/kg) had a significant reduction to 74.4% in testis weight compared control. Epididymal sperm number was reduced to 5% of control and sperm motility was decreased to 3% of control. Moreover, high dose group showed sterility mated with normal females. Biochemical study showed that the BrdT expression is dose-dependantly decreased in these mice model. Low dose (1mg/kg, 2mg/kg and 3mg/kg) groups had no difference in testis weight and fertility but sperm number and motility was decreased compared with control. After withdrawal the drug about 25 days, the high dose group males had recovery of the fertility, testis weight and sperm number.
Limitations, reasons for caution: Mice treated with NHWD-870 showed decreased level of testosterone in serum. High dose drug also lead to decreased body weight after 6 weeks treatment.
Wider implications of the findings: By optimizing the structure of the NHWD-870, it could be possibly used in human as an oral medication for reversible male contraception.
Trial registration number: Not applicable

P-018 Live birth rate of patients where sperm selected using microfluidic technique in high DNA fragmentation index sperm samples

F. Khan[1], K.D. Nayar[1], G. Kant[1], M. Singh[1], S. Mishra[1], S. Gupta[1], R. Bhattacharya[1], K.D. Nayar[1]

[1]Akanksha IVF Centre- Mata Chanan Devi Hospital, Reproductive Medicine, New Delhi, India

Study question: Does microfluidic sorting technique help in increasing live birth rate in patient with high DNA fragmentation index (DFI) sperm samples?
Summary answer: Sperm selected by microfluidic sorting are associated with significant increase in live birth rate, clinical pregnancy rate and reduced miscarriage rate.
What is known already: DNA damage is unrecognisable in living sperm prior to insemination and an increased sperm DNA fragmentation index has been associated with lower fertilization rates, impaired embryo development and reduced pregnancy rates. Standard semen processing techniques are associated with centrifugation, which may induce reactive oxygen species and DNA damage. Microfluidic systems are capable of working with small volume samples and have high sensitivity and low response time. This technique helps to improve the efficiency of sample preparation, enable consistency in embryo culturing and reduce human error. It has been demonstrated that microfluidic technique could provide sperm with significantly reduced DNA damage.
Study design, size, duration: A prospective randomised control study was conducted from 1st August 2017 to 31st December 2018. One hundred and nighty eight patients were randomised by computer generated list and divided into 2 groups. Group A (n=100) , in which sperm were processed by microfluidic sperm sorter while in group B (n=98), sperm were processed by density gradient technique and morphologically normal motile sperm were injected by Intracytoplasmic sperm injection (ICSI) technique in all mature oocytes.
Participants/materials, setting, methods: The study period included all normozoospermic patients with high DNA fragmentation index (>25%) while oligospermic, asthenozoospermic samples, patients with poor ovarian reserve and advanced age were excluded from the study. All A grade embryos were vitrified and transferred in frozen embryo transfer cycle. Both groups were compared on the basis of fertilisation rate, day 3 grade A embryo development rate, clinical pregnancy rate, miscarriage rate and live birth rate.
Main results and the role of chance: Cycle characteristics (female age, length of stimulation, gonadotrophin dose, number of oocytes and number of transferred embryos) were similar in both groups.

Between the two groups, there was a significant increase observed in group A over group B in day 3 grade A embryo development rate (60% vs. 38%, p-0.003), clinical pregnancy rate (62% vs. 41%, p-0.004) and live birth rate (46% vs. 29%, p-0.011), while a significant decrease in miscarriage rate (12% vs. 25%, p-0.028). On the other hand there was no statistical difference observed in fertilisation rate (82% vs. 78%, p-0.80).
Limitations, reasons for caution: Larger randomised control studies are needed to strengthen these results.
Wider implications of the findings: We have demonstrated that sperm sorted by microfluidic not only helps in selection of sperm with better DNA integrity but also increased live birth rate. Using it in routine practice can help in reducing the negative effect of reactive oxygen species and thus improve pregnancy rate and live birth rate.
Trial registration number: MCDH/2017/35

P-019 Male dietary fat intake and fecundability: a preconception cohort study

L.A. Wise[1], A. Wesselink[1], S. Willis[1], E. Mikkelsen[2], K. Tucker[3], A.S. Dam Laursen[2], K. Rothman[1], E. Hatch[1]

[1]Boston University School of Public Health, Department of Epidemiology, Boston, U.S.A. ;
[2]Aarhus University Hospital, Department of Clinic Epidemiology, Aarhus, Denmark ;
[3]University of Massachusetts at Lowell, Biomedical & Nutritional Sciences, Lowell, U.S.A.

Study question: To what extent does male dietary fat intake influence fecundability?
Summary answer: We observed little overall association between intakes of total fat, and most fat subtypes, and fecundability.
What is known already: Higher intakes of saturated fat and *trans* fatty acids have been associated with poor semen quality and low testosterone levels, whereas higher intakes of omega-3 fatty acids have been associated with improved semen quality in some studies. There have been few preconception cohort studies of male diet and fecundability.
Study design, size, duration: Pregnancy Online Study (PRESTO) is an ongoing North American prospective preconception cohort study. Analyses were restricted to 446 couples attempting conception for ≤6 cycles at enrollment during 2013-2020.

Participants/materials, setting, methods: Men aged ≥21 years completed an online baseline questionnaire on demographic, medical, and anthropometric factors. Ten days after enrollment, they completed a validated food frequency questionnaire (DHQ II). Their female partners completed bimonthly follow-up questionnaires for up to 12 months or until pregnancy. We used proportional probabilities regression to estimate fecundability ratios (FR) and 95% CIs for associations of % energy from total fat and fat subtypes with fecundability, adjusting for energy and other covariates.

Main results and the role of chance: Intakes of total fat, saturated fat, mono-or poly-unsaturated fat, or trans fat intake were not appreciably associated with fecundability. FRs (95% CIs) comparing the top vs. bottom quartiles of dietary fat intake were 1.00 (0.73-1.36) for *trans* fatty acids, 1.15 (0.84-1.57) for omega-3 fatty acids, 0.78 (0.56-1.08) for omega-6 fatty acids, and 1.23 (0.93-1.64) for the ratio of omega-3 to omega-6 fatty acids.

Limitations, reasons for caution: We observed little overall association between male intake of total fat, and most fat subtypes, and fecundability. Weak associations were seen for intakes of omega-6 fatty acids and the ratio of omega-3 to omega-6 fatty acids, though associations were imprecise.

Wider implications of the findings: These data contribute to the limited existing literature on the association between male diet, specifically fat intake, and fecundability. Although numbers were small, these findings indicate that male dietary intake of fat is not a strong determinant of fecundity.

Trial registration number: NICHD grants R21HD072326, R01 HD086742, and R03 HD090315

P-020 L-carnitine in seminal plasma correlated with semen parameters and sperm DNA fragmentation

L. Ye[1], W. Huang[1], M. Song[1], J. Han[1], Y. Peng[1], T. Wu[2], Y. Zeng[3,4]

[1]*Shenzhen Zhongshan Urology Hospital, Department of Andrology and Urology, Shenzhen, China ;*
[2]*Shenzhen Zhongshan Urology Hospital, Department of Reproductive Immunology, Shenzhen, China ;*
[3]*Shenzhen Zhongshan Urology Hospital, Fertility Center, Shenzhen, China ;*
[4]*Shenzhen Zhongshan Urology Hospital, Shenzhen Key Laboratory for Reproductive Immunology of Preimplantation, Shenzhen, China*

Study question: Is there any relationship between L-carnitine in seminal plasma and sperm count, motility, morphology and DNA integrity?

Summary answer: L-carnitine in seminal plasma was positively corelated with sperm count, progressively motile sperm, morphologically normal sperm as well as sperm DNA fragmentation.

What is known already: L-carnitine, presenting in testis and epididymis, is involved in sperm maturation and it is used as antioxidants in therapy of idiopathic male infertility. Evidence showed that oral L-carnitine can improve sperm parameters including total sperm count, concentration, progressive motility, vitality, and morphology in infertile men. Moreover, recently publications indicated that L-carnitine preserves the sperm membrane and DNA integrity. But limited researches investigated the relationship of L-carnitine in semen and sperm parameters as well as sperm DNA fragmentation.

Study design, size, duration: Data of semen analysis from January 2017 to December 2019 were involved and divided into normozoospermic group and non-normozoospermic group according to WHO guideline. Total content of L-carnitine in seminal plasma, sperm DNA fragmentation index (DFI) and semen parameters including sperm numbers, motility and morpology were retrospectively analyzed. For all the included study subjects, the leukocytes count in semen less than 1×10^6/ml.

Participants/materials, setting, methods: Sperm count and motility were analyzed by computer assisted sperm analysis (CASA) system, while sperm morphology was examined after Diff-Quik rapid staining procedure. L-carnitine in seminal plasma was determined by high-performance liquid chromatography. DFI was calculated from sperm chromatin structure analysis (SCSA). Content of L-carnitine and DFI of normozoospermic group abnormal group and was compared by Mann-whitney U test. Bivariate correlation analysis was used to explore the relationship between L-carnitine and sperm paraments and DFI.

Main results and the role of chance: A total of 4437 subjects were enrolled in the study and normozoospermic group contained 2591 cases while non-normozoospermic group contained 1846 cases. Median value of L-carnitine in seminal plasma was significantly higher in normozoospermic group [458.78(439.92) nmol per ejaculate] than that in non-normozoospermic group [347.58(447.61) nmol per ejaculate] (P<0.001). Mean value of DFI in normozoospermic group was6.97% (6.07%), which was lower than DFI in abnormal group [14.51% (13.41%)] (P<0.001) Content of L-carnitine in semen was positively corelated with total sperm count, percentage of progressively motile sperm and percentage of morphologically normal spermatozoa, whose coefficient of correlation was 0.580 (P<0.001), 0.150 (P<0.001) and 0.131 (P<0.001) respectively. Moreover, L-carnitine in seminal plasma was negatively corelated with DFI, whose coefficient of correlation was 0.065 (P<0.001).

Limitations, reasons for caution:
It is a single-center study and the above results were from retrospective analysis

Wider implications of the findings:
The above results can be used as references in oral L-carnitine of treatment in improving semen paraments and the choice of antioxidants in reducing sperm DNA fragmentation.

Trial registration number: Not Applicable

P-021 The relationship between seminal leukocytes and oxidative stress markers in semen

S. Kuroda[1], T. Takeshima[1], Y. Yumura[1]

[1]*Yokohama City University Medical Center, Department of Urology- Reproduction center, Yokohama, Japan*

Study question: Does leukocyte concentration in semen and semen parameters correlate with oxidative stress markers?

Summary answer: Seminal leukocytes concentration was strongly correlated with ROS level in semen, and ORP was more negatively correlated semen parameters than ROS.

What is known already: Reactive oxygen species (ROS) in semen has been reported to have negative effect to male fertile capacity, and recent studies reported the efficacy of oxidation-reduction potential (ORP) which reflects the balance of oxidants and antioxidants in semen. The source of ROS in semen is considered as immature spermatozoa and seminal leukocytes though the detail is still unknown. Pyospermia, globally defined as the presence of more than one million leukocytes in 1 mL of semen, is considered as an indicator for genital infection and inflammation.

Study design, size, duration: Between November 2018 and July 2019, 50 infertile males who visited our hospital were enrolled. All patients underwent semen analysis and measurement of ROS and ORP levels and the concentration of leukocytes in semen. The correlation between these values were analyzed retrospectively.

Participants/materials, setting, methods: ROS level in semen was measured using Monolight 3010™ Luminometer. Before and after adding 40 mL of 100 mmol/L luminol to 500 mL of semen sample, the subtraction of the integrated chemiluminescence was measured between 0 and 200 seconds. ORP level per sperm concentration (sORP) was measured using MiOXSYS System™. The concentration of leukocytes were evaluated using myeloperoxidase staining (Endtz test). The relationships between these values and semen parameters were evaluated using non-parametric correlation analysis.

Main results and the role of chance: The average patient's age was 37.1 (20-53). ROS level was positively correlated with leukocyte concentration (ρ=**0.646, p<0.001)** although sORP didn't show significant correlation. Both ROS level and sORP showed negative correlation with sperm concentration and motility significantly, although the Spearman correlation coefficient was higher in sORP than in ROS (ρ= -0.873 vs -0.425, -0.547 vs -0.228, respectively). sORP was also negatively correlated with straight velocity and mean amplitude of lateral head displacement (mean ALH). When the positive cut-off value of ROS level was set 4332 RLU as previously reported, ROS was positive more than 1.1×10^5/ml of leukocytes in semen according to ROC curve (AUROC= 0.862) .

Limitations, reasons for caution: This study is retrospective and single center analysis with small number.

Wider implications of the findings: Seminal leukocyte was considered as a source of ROS in semen. ROS was positive even under the global definition of pyospermia, thus fewer leukocytes than definition may have adverse effects to sperm quality through ROS generation. ORP have potential as keener biomarker to reflect the sperm quality than ROS.

Trial registration number: not applicable

P-022 Does Testicular Sperm Aspiration have a place in the Management of Non-Obstructive Azoospermia Patients?

A. Majzoub[1], K. Khalafalla[1], H. Elbardisi[1], S. Alsaid[1], M. Arafa[1]

[1]Hamad Medical Corporation, Urology, Doha, Qatar

Study question: Are there any clinical predictors that could help in selecting non-obstructive azoospermia (NOA) candidates for testicular sperm aspiration (TESA)?
Summary answer: TESA may be offered for patients with a serum FSH level <7.5IU/L and average testicular size >7.75ml
What is known already: Microsurgical testicular sperm extraction (m-TESE) is the gold standard surgical sperm retrieval technique allowing magnified evaluation and biopsy of a large area of testicular tissue thereby increasing the overall sperm retrieval rate (SRR). However, while a small quantity of testicular tissue is presumably extracted during m-TESE, the procedure can have detrimental effects on testicular function evidenced by the resulting temporary or permanent state of hypogonadism postoperatively. Furthermore, microTESE is expensive, time-consuming and requires a skilled microsurgeon who may not be available in certain places. These facts advocate that TESA may still be of value in the management of NOA patients.
Study design, size, duration: This was a retrospective study of 394 patients with NOA who underwent their first trial of surgical sperm retrieval over a period of 5 years. Patients with NOA based on the presence of one or more of the following: 1) histopathology denoting defective spermatogenesis; 2) high gonadotropins; 3) scanty number of sperm retrieval; 4) genetic abnormality were included. We excluded patients with proven obstructive azoospermia or who performed surgery due to causes other than azoospermia.
Participants/materials, setting, methods: Data including age, testicular size, serum hormones, genetic tests and histopathologic results were collected. All patients underwent a staged retrieval procedure starting with TESA and proceeding to m-TESE. Multivariate logistic regression analysis was used to assess the impact of different factors on the outcome. ROC curve was performed to identify the cut-off value for significant variables. The likelihood for positive TESA-SRR was calculated for each significant variable and for all variables combined using regression analysis.
Main results and the role of chance: TESA was the sole procedure in 31% of patients while two-thirds of patients underwent m-TESE. Overall, SRR with TESA was 26.7% while with m-TESE was 42.3%. Patients with positive TESA had significantly larger testicular size (10.39 ± 0.6 vs. 7.15 ± 0.3, p<0.001) and significantly lower FSH (6.44 ± 0.7 vs. 14.1 ± 0.8) and LH levels (4.03 ± 0.3 vs. 7.15 ± 0.4 (p<0.001 for both) than those with a negative TESA. The highest SRR was observed in men with hypospermatogenesis (52.6%) followed by Maturation arrest (34.2%), Sertoli cell only (13.2%) and tubular hyalinization (0%) (p<0.001). Multivariate analysis revealed that only testicular size (OR 1.084, 95%CI 1.001-1.174, p=0.046) and serum FSH (OR 0.924, 95%CI 0.863-0.989, p=0.024) had a significant impact on SRR with TESA. A testicular size cut-off of 7.75ml (sensitivity 62.9%, specificity 63.4%, AUC=0.674) and FSH cut-off of 7.5IU/L (sensitivity 64.6%, specificity 64%, AUC=0.718) were the best predictors of TESA SRR. TESA was positive in 39.5% of patients with FSH < 7.5 IU/L (OR 3.247, p< 0.001), 34.6% of patients with testicular size > 7.75 ml (OR 2.916, p<0.001) and 42.5% of patients with both FSH < 7.5 IU/L and testes size > 7.75 ml (OR 3.419, p<0.001)
Limitations, reasons for caution: The main limitations are the retrospective design of the study.
Wider implications of the findings: Investigating the predictors for positive SRR with TESA may provide important prognostic evidence that can be used during patient counselling to aid the couple in making sound treatment decisions.
Trial registration number: NA

P-023 The semen microbiome and the impact on sperm function and male fertility: a systematic review and meta-analysis

L. Farahani[1,2], T. Tharakan[2,3], T. Yap[4], J. Ramsay[3,5], C. Jayasena[2,5], S. Minhas[3]

[1]Imperial College Healthcare NHS Trust, Obstetrics & Gynaecology, London, United Kingdom ;
[2]Imperial College, Metabolism- Digestion and Reproduction, London, United Kingdom ;
[3]Imperial College Healthcare NHS Trust, Urology, London, United Kingdom ;
[4]Guy's and St Thomas' NHS Foundation Trust, Urology, London, United Kingdom ;
[5]Imperial College Healthcare NHS Trust, Andrology, London, United Kingdom

Study question: What constitutes the semen microbiome (SM) and what is the impact of the semen microbiome on male fertility and semen parameters?
Summary answer: Specific bacterial species are associated with worsening sperm quality, whilst others are protective, but the association with infertility is uncertain
What is known already: Male factor is attributable in up to 50% of cases of infertility. It is widely known that bacterial infection can impair sperm function. In-vitro studies demonstrate that bacteria can reduce sperm function through mechanisms such as motile sperm agglutination or induction of apoptosis. The use of next-generation sequencing(NGS) techniques has provided a better understanding of the microbiome and has led to the emergence of new therapeutic approaches, and recent studies have highlighted the importance of the changes in the microbiome(dysbiosis) to female reproductive health. Several emerging studies have investigated the impact of the semen microbiome on sperm function and fertility.
Study design, size, duration: A systematic search was conducted in accordance with the Preferred Reporting Items for Reviews and Meta-analysis (PRISMA) statement. The systematic review was registered with PROSPERO (ID CRD42019124483). The databases MEDLINE, OVID and PubMed were searched to identify English language studies related to the identification of bacteria in the semen of infertile and fertile men, between 1st January 1999 and 15th March 2019. Two independent investigators screened abstracts and selected studies and extracted data for meta-analysis
Participants/materials, setting, methods: 54 studies were included, with 50811 subjects. Observational studies were included that identified the seminal microbiome in male subjects, and it's association with fertility and semen parameters. We included studies that analysed semen according to the 1999 or 2010 WHO manual, and where bacteria were identified using NGS, culture or polymerase chain reaction (PCR). We excluded animal studies. The National Institute of Health quality assessment tool was used to grade the studies.
Main results and the role of chance: NGS enabled the detection of greater variety of bacterial genera than culture/PCR. The SM was reported to be rich and diverse in both fertile and infertile men. It is uncertain if the microbiome differs between these groups as only 1 NGS paper studied this (n=77 participants), and found no difference.

Three of the four NGS studies reported clustering of the seminal microbiome with a predominant species. All three studies reported that Lactobacillus and Prevotella were dominant in respective clusters. Lactobacillus was associated with improvements in semen parameters, and had a protective effect on quality. Conversely Prevotella appeared to exert a negative effect on sperm quality.

Bacteriospermia negatively impacted sperm concentration (difference in means(MD) -15.6,95%CI -22.65to-8.65) and progressive motility(PM)(MD -8.105,95%CI -13.568to-2.642). There was an increased prevalence of Ureaplasma urealyticum (UU) in infertile men(OR 2.25, 95% CI 1.47-3.46). UU negatively impacted concentration (MD -13.85,95%CI -20.93to-6.67) and morphology (MD -2.82,95%CI -3.546to-2.1). There was no difference in the prevalence of chlamydia trachomatis (CT) between fertile and infertile men(OR 1.11, 95%CI 0.75-1.65), and CT had no significant impact on routine semen parameters(concentration:MD -8.723,95%CI -19.255to1.809,PM:MD -1.727,95%CI -5.567to2.112, morphology:MD 0.449,95%CI -1.601to2.5). Enterococcus faecalis(EF) negatively impacted total motility(MD -11.03,95%CI -17.845to-4.22), and Mycoplasma hominis(MH) negatively impacted concentration, PM and morphology(p<0.05).
Limitations, reasons for caution: The quality of the evidence ranged from fair to low quality, and although 50,811 total subjects were studied, the numbers in the NGS studies were small (n=385). There was a high degree of heterogeneity in some of the individual meta-analyses and therefore caution must be observed when interpreting the results.
Wider implications of the findings: NGS is a superior method of characterizing the SM. UU, EF, MH and Prevotella negatively impact sperm, whereas Lactobacillus appears to protect sperm, paving the way for novel therapies (e.g.probiotics). The evidence regarding the impact of microbiome on fertility is inconclusive. Couples should be informed that further studies are needed.
Trial registration number: not applicable

P-024 Is there an endocrine contribution to the sexual dysfunction seen in end stage renal disease patients ?

S. Al Said[1], H. Elbardisi[1], K. Khalafalla[1], M. Arafa[1], A. Majzoub[1]

[1]Hamad Medical Corporation, Urology, Doha, Qatar

Study question: Does hormonal disturbance in end stage renal disease patients affect their sexual function ?
Summary answer: Endstage renal disease is commonly associated with sexual dysfunction that is more likely to be attributed to organic causes rather than solely to endocrine disturbances
What is known already: Male sexual dysfunction is commonly prevalent in patients with end stage renal disease (ESRD) and has been partly attributed to the concurrent state of hyperprolactinemia and hypogonadism, often observed in this patient population. The aim of this study was to evaluate the hormone profile and sexual function of ESRD patient, using validated questionnaires, in attempt to explore this association.
Study design, size, duration: This was a prospective study which included 98 patients with end stage renal disease (ESRD) who followed in the outpatient department of a tertiary medical centre over a period of 1 year. Patients receiving treatment for hyperprolactinemia or those known to have an endocrine disorder were excluded in addition to patients receiving medical or surgical treatment for erectile dysfunction (ED) or premature ejaculation (PE).
Participants/materials, setting, methods: Full history details with complete physical general and genital exam was conducted on the included patients. After filling the international index for erectile function-5 and the Arabic index for PE questionnaire, morning serum samples were taken from patients to measure testosterone and prolactin levels. Descriptive statistics was used to report frequency or means of variables. Chi-square test was used to examine associations between categorical variables. P<0.05 was considered statistically significant.
Main results and the role of chance: Out of the 98 ESRD patients, 72 (73.6%) were treated with hemodialysis, 13 (13.2%) with peritoneal dialysis and 13 (13.2%) with medical treatment only. Diabetes mellitus was observed in all patients (type 1, 52%; and type 2, 48%), while hypertension, coronary heart disease and dyslipidemia were detected in 97.1%, 34.3% and 25.5%, respectively. The mean age, serum testosterone and prolactin levels were 52.4 ± 12.1 years, 12.95 ± 6.5 nmol/L and 514.2 ± 592.8mIU/L. Results of the PE index questionnaire revealed that 86 (87.7) patients had PE, 9 (9.1%) probable PE and 3 (3.1%) no PE. With IIEF-5, ED was detected in 96 patients; it was severe in 23 (23.5%), moderate- severe ED in 29 (29.4%), mild-moderate ED in 30 (30.4%) and mild in 14 (14.7%). Patients were divided into 2 groups, one according to prolactin levels (normal and high) and other according to testosterone level (low and normal). 55 patients had high prolactin while 33 had low testosterone levels. No significant differences were observed in IIEF or PE index levels between patients with low/normal testosterone and normal/high prolactin.
Limitations, reasons for caution: This is a single center study.
Wider implications of the findings: Since hormonal disturbance doesn't affect sexual dysfunction, definitive treatment for sexual dysfunction should be started including medical or surgical options.
Trial registration number: NA

P-025 Prospective follow-up of semen parameters in spinal cord-injured patients (FertiSCI): a pilot study

C. Chalas[1], L. Jilet[2], V. Drouineaud[1], C. Patrat[1], J.P. Wolf[1], F. Giuliano[3]

[1]Hôpital Cochin-Port Royal, Biology of Reproduction, Paris Cedex 14, France ;
[2]URC/CIC Paris Descartes Necker Cochin, Biostatitics, Paris, France ;
[3]Hopital R. Poincare APHP, Neuro-urology, Garches, France

Study question: Are men with spinal cord injury (SCI) at risk for semen parameters impairment overtime according to the lesion characteristics and associated genital inflammatory state?
Summary answer: Neither genital inflammatory state, completenesslevel of the lesion,age nor time post SCI, seemed to exert any influence on semen quality over time.
What is known already: Spinal cord injury (SCI) primarily affects young men who have not experienced fatherhood yet. SCI often results in erectile dysfunction and anejaculation during coïtus. Semen parameters are often impaired in SCI patients mostly spermatozoa motility, vitality and typical forms associated with an elevated concentration of leukocytes. SCI patients are at high risk of urinary tract infections and positive spermoculture generating an inflammatory syndrome (IS) and exposing spermatozoa to oxidative stress (OS). Fertility is a major concern in this population and many of them fear that the delay post-SCI can negatively impact sperm quality.
Study design, size, duration: 35 SCI patients aged from 18 to 60 years old able to ejaculate by masturbation or in response to penile vibratory stimulation (PVS) have been enrolled. A prospective longitudinal study was conducted over 18 months with 4 visits to collect medical information focusing on uro-genital tract and concomitant treatments. At each visit a semen analysis was performed with the evaluation of seminal IS and OS. Thirty-five SCI patients have been included.
Participants/materials, setting, methods: Semen analysis was conducted according to WHO recommendations. IS was quantified through granulocyte concentration evaluated by leucoscreen® test and considered positive when >1million/ml and seminal plasma elastase measured by ELISA with a 500 ng/ml positive threshold. OS was assessed using the Tunel assay methodology and analyzed by flow cytometry to measure spermatozoa DNA fragmentation with a 40% positive threshold. Data were compared using Wilcoxon signed rank and the Mann-Whitney-Wilcoxon tests.
Main results and the role of chance: SCI patients mean age of was 29.4 ± 6.4 years with mean age at SCI 21.8 ± 8.3 years. Semen concentration was normal (155.1±231.2 million/ml) associated to a decrease in progressive motility (13.6±12.4%) and vitality (22.7±19.3%) and an increase in morphological abnormalities (typical forms 11.5±11.2%). Mean round cell semen concentration was increased (38.3±37.6 million/ml) as well as granulocyte concentration (46.4±121.1 million/ml). IS and OS were highly positive with a median elastase quantified at 477.4 ng/ml and the DNA fragmentation at 50%. Semen parameters were compared between patients i) positive and negative for IS and OS and ii) in absence or presence of a urogenital i.e. urinary and/or seminal tract (UGI) infection. iii) taking into account the lesion level, the completeness of the lesion, the time elapsed since SCI and age at injury. We could not find any difference in semen parameters in the presence of IS and OS nor when UGI was present. Moreover, neither completeness nor level of the lesion nor time post SCI nor age at injury did seem to influence semen quality. Semen parameters at the first and the last visit were compared in 17 patients and showed no change over time.
Limitations, reasons for caution: The study was conducted in a small sample. Only 28 semen samples were collected at first visit (V1) versus 11 at V4 indicating a high number of patients lost of follow-up. In some cases planed semen analysis has been hindered because of the decrease in semen volume characteristic of SCI.
Wider implications of the findings: Within the limits of the study, there was no significant decline in semen quality overtime. Even preliminary, these findings are essential to reassure SCI men about their future fertility. The present study is not in favor of routine sperm freezing for fertility preservation in SCI patients.
Trial registration number: NCT02144558

P-026 The Efficacy of Repeat Micro TESE after Failed First Attempt in Men with Nonobstructive Azoospermia

E. Bakircioglu[1], O. Ozman[2], S. Tosun[3], B. Numan[3], C. Sami[3], O. Cenk[3]

[1]Istanbul Florence Nightingale Hospital, Urology, Istanbul, Turkey ;
[2]Health Sciences University- Gaziosmanpaşa Taksim Education and Research Hospital, Urology, Istanbul, Turkey ;
[3]Istanbul Florence Nightingale Hospital, In Vitro Fertilization Center, Istanbul, Turkey

Study question: What is the efficacy of repeat micro-TESE in men with non-obstructive azoospermia who failed the first micro-TESE?
Summary answer: Repeat micro-TESE was enabled retrieved sperm in 18.4% of men with NOA who failed the first micro-TESE.
What is known already: Testicular sperm extraction combined with ICSI is the only way for the patients with non-obstructive azoospermia to have their own genetic offspring. Micro-TESE promises high rates of surgical sperm retrieval compared to methods such as testicular sperm aspiration and conventional TESE.

Salvage micro-TESE operation showed successful sperm recovery after failed conventional TESE in many studies in the literature.
Study design, size, duration: This was a retrospective cohort study of 125 men who failed the previous micro-TESE in other institutions that underwent repeat micro-TESE between November 2014 and July 2018. Clinical parameters of the patients were compared for the success of sperm recovery between the patients who were successful for sperm recovery and who have not.
Participants/materials, setting, methods: All of the patients underwent repeat micro-TESE operation. The age of the patients, duration of infertility, serum FSH and total testosterone levels and testicular volumes were compared between the patients who have successful sperm recovery and who have not.
Main results and the role of chance: Sperm was successfully recovered in 23 of 125 (18,4%) men with repeat micro-TESE. The age of patients (33.9±4.7,33.7±5.5; P=0.86), the duration of infertility (7.1±4.3, 6.4±3.5; P=0.47) serum FSH (22.0±13.8, 15.4±13; P=0.29) and total testosterone level (3.4±1.1, 3.1±1.6; P=0.37) did not show a statistical difference between men who have successful sperm recovery and who have not. However, testicular volume was significantly lower in men who have successful sperm recovery (8.2±5.4 ml) compared to men who have not (11.3±5.3 ml, p=0.01). Seven of 14 (50%) men who have diagnosed Klinefelter syndrome (KS) sperm recovery was successful with repeat micro-TESE.
Limitations, reasons for caution: It is a retrospective cohort study. Official records of the micro-TESE operations that the patients had previously were insufficient.
Wider implications of the findings: Our cohort is the largest repeat micro-TESE series in patients who have non-obstructive azoospermia with a failed first attempt. The chance of sperm recovery may enable valuable information for the patients before the decision to repeat micro-TESE.
Trial registration number: Not applicable

P-027 Tubular ectasia of epididymis and rete testis among azoospermia patients

H. Nguyen[1,2], H.H.T. Nguyen[1], C.K. Trinh[1], T.A. Nguyen[1], V.H. Dinh[1]

[1]Andrology and Fertility Hospital of Hanoi- Vietnam, Andrology department, Hanoi, Vietnam ;
[2]Vinmec International General Hospital, Andrology department, Hanoi, Vietnam

Study question: To identify the rate of tubular ectasia of epididymis and rete testis (TEERT) in infertile men with obstructive and non-obstructive azoospermia (OA and NOA).
Summary answer: Rates of TEERT in patients with OA and NOA were 87.8% and 22.5%, respectively.
What is known already: TEERT is a condition mainly relating to the obstruction of seminal tract. With high resolution ultrasound and the routine use of scrotal ultrasonography, this condition is increasingly diagnosed. However, it is often unclear about the clinical significance of this diagnosis.
Study design, size, duration: This monocentric, retrospective study was conducted on 203 infertile men with azoospermia who visited Andrology and Fertility Hospital of Hanoi (AFHanoi) from November 2017 to October 2019 for seeking treatment on infertility.
Participants/materials, setting, methods: Patients were chosen to take part in the study if they had information of clinical examination, serum follicle-stimulating hormone (FSH), testosterone, scrotal ultrasonography performed at AFHanoi. Participants had also to undergoing any of these sperm retrieval procedures i.e. PESA, TESE and micro-TESE in the same hospital.
Main results and the role of chance: There were 123 (60.6%) patients with OA and 80 (39.4%) patients with NOA. Rates of tubular ectasia of epididymis and rete testis in the whole sample was 62.0% (126/203 men). In men with OA, rate of TEERT was 87.8% (108/123 men), whereas in men with NOA this rate was 22.5 (18/80 men). Interestingly, among those with tubular ectasia almost all cases had the condition observed in epididymis (106/108 cases, 98.0%). However, only 2 cases (2%) had the condition observed in rete testis. As expected, sperm retrieval rate in those with TEERT was as high as 96% (121/126 men).
Limitations, reasons for caution: This study is a retrospective cohort study with all associated inherent biases. The findings of this study should be confirmed with further prospective study.
Wider implications of the findings: Our study shows that TEERT is highly prevalent in infertile men with OA and those with TEERT are much likely having their sperm retrieved by a sperm retrieval technique. This finding suggests the predictive value of TEERT in the prediction of OA and sperm retrieval.
Trial registration number: not applicable

P-028 Bicarbonate-dependent alkalinization of acrosomal pH occurs during human sperm capacitation

"Abstract withdrawn by the authors"

P-029 Efficacy of Magnetic Activated Cell Sorting (MACS) in Sperm Selection for Intracytoplasmic Sperm Injection (ICSI)

T.T.H. Tran[1], T.T.C. Bach[1], V.T. Phung[1], T.T. Tran[1], T.L. Dinh[1]

[1]Hospital of Post and Telecoms, Assisted reproductive technology center, Hanoi, Vietnam

Study question: This study investigated if the application of MACS would have a critical effect on ICSI outcome, especially with patients having multiple unsuccessful IVF cycles.
Summary answer: The results of this study suggested favorable influence on patients with multiple failed IVF cycles and highly promising for others.
What is known already: MACS is reported as a possible procedure to help increase ICSI outcome by using annexin V to separate apoptosis sperms. The separation of apoptosis sperms is believed to result in a positive change in sperm quality.
Study design, size, duration: 114 patients were enrolled in this study between February 2018 to January 2020. We analysed the outcome of 52 multiple returning cycle couples who has undergone one MACS/ICSI cycle (group 1) and their most recent non-MACS/ICSI cycle (group 2). We also accessed the outcome of 62 couples who has only has one cycle of IVF done (group 3).
Participants/materials, setting, methods: Group 1 and 2 comprised of 52 multiple returning cycle couples who has undergone one MACS/ICSI cycle and one non-MACS/ICSI cycle. 62 participants in our group 3 has only one IVF cycle and has specifically requested for MACS/ICSI to be performed. In all MACS/ICSI cycles, ejaculated semen was undergone density gradient centrifugation in combination with MACS. Collected sperms were used for routine ICSI. T-Test and Chi-square test were applied.
Main results and the role of chance: Group 1 has higher fertilization rate (67%) than group 2 (59%). Group 2 with non-MACS procedures saw 16 embryo tranfers for the first time and 04 for the second, performed on X couples with 8 couple having no embryos; cumulatively, positive beta-HCG rate was 13%. Whereas in group 1, all but 1 couples had embryos; 19 couples have had embryo transferred, resulted in 45 % positive beta-HCG rate; In group 3, the FR and positive beta-HCG rate respectively was 78% and 51%. In conclusion, there were significant differences in the result of group 1 and 2. For group 3 where patients requested to perform MACS , the result was also very promising.
Limitations, reasons for caution: The sample size of this study participants is small. Further studies with more control groups with sperm and eggs quality analytics is needed to confirm our methodology.
Wider implications of the findings: According to this study, the application of MAC/ICSI is a novel and easy to perform clicician technique with promising results.
Trial registration number: Not applicable

P-030 Severe male factor affects the blastocysts ploidy status

E. Zhylkova[1], O. Feskov[2], O. Somova[3], O. Zozulina[4], A. Feskova[5]

[1]Centre of Human Reproduction Sana-Med, Genetic laboratory, Kharkiv, Ukraine ;
[2]Center of Human Reproduction "Clinic of Professor Feskov A.M.", IVF department, Kharkov, Ukraine ;
[3]Center of Human Reproduction "Clinic of Professor Feskov A.M.", IVF Department, Kharkiv, Ukraine ;
[4]Center of Human Reproduction "Clinic of Professor Feskov A.M.", IVF Department, Kiev, Ukraine ;
[5]Kharkiv National Medical University, Department of Obstetrics- Gynecology and Pediatric Gynecology, Kharkiv, Ukraine

Study question: Does the severe male factor affect the blastocysts ploidy status and IVF outcome?
Summary answer: Severe male factor of infertility affects the blastocysts' aneuploidy. Sperm parameters affect blastocyst morphology. The IVF outcome does not depends on severe pathospermia.
What is known already: Male factor of infertility is responsible for up to 60% of fertility problems worldwide. The development of intracytoplasmic sperm injection (ICSI) has allowed patients with severe oligozoospermia to have children. The question about the effect of severe male infertility on embryo aneuploidy and IVF results is still discussable. Almost 60% of the blastocysts in IVF are aneuploid. Existing data regarding the relationship between embryo morphology and its ploidy are conflicting.
Study design, size, duration: The effect of severe male factor of infertility on the early embryo development was studied. The correlation of sperm parameters with the morphology of blastocysts and PGD and IVF outcomes was examined. The study's protocol was approved by the Center's IRB.
Participants/materials, setting, methods: Totally the DNA samples of 406 preimplantation blastocysts from 117 men diagnosed with severe male factor of infertility and normal karyotype 46,XY were examined using the method of next generation sequencing (NGS). ICSI/IMSI procedure was done. The rates of blastocysts euploidy and IVF outcome were compared with 299 biopsied blastocysts from 67 patients with normal sperm parameters. A chi-squared test and Spearman coefficient were used to perform statistics analysis.
Main results and the role of chance: The rate of blastocysts aneuploidies was significantly higher in group of patients with severe male factor of infertility comparing with the control group (51.1% vs. 33.8% respectively, $\chi^2 = 6.125$, $\chi^2_{critic.} = 3.841$, $P = 0.014$). There was a significant positive correlation between the blastocysts euploidy rates and sperm concentration ($r_s = 0.16$, $P < 0.05$). A significant positive correlation between sperm motility and blastocysts morphology was proved ($r_s = 0.17$, $P < 0.05$). The transfer of one euploid blastocyst was performed in each case for patients in both studied groups. There was no significant difference in IVF outcomes in both groups. The clinical pregnancy rates were 53.8% and 63.2% in group of patients with severe male factor of infertility and in control group respectively (P>0.05).
Limitations, reasons for caution: Blastocyst biopsy can be carried out for the embryos with the high quality of morphology.
Wider implications of the findings: *The* formation of the blastocyst and embryo implantation may be blocked at various stages of development. Performing the PGD test using the high resolution techniques for patients with severe pathospermia results in high IVF outcomes. The necessity of PGD for patients with severe male infertility is proved.
Trial registration number: No number

P-031 Vitamin D supplementation improves the Leydig Cell function in infertile men with persistent vitamin D insufficiency

R. Holt[1]

Study question: Can vitamin D supplementation improve the Leydig Cell function in infertile men?
Summary answer: Yes, high-dose vitamin D supplementation had a beneficial effect on Leydig Cell function in infertile men with low vitamin D and high PTH.
What is known already: Vitamin D is important for calcium homeostasis and bone health, but the spectrum of vitamin D related effects has expanded in recent years. All actions of vitamin D are mediated by the interaction between activated vitamin D (calcitriol) and the vitamin D receptor (VDR) which is expressed in human Leydig cells and thus makes a direct effect of vitamin D on steroidogenesis plausible. Analyses of VDR knock-out (KO) mice revealed impaired reproductive and endocrine function compared with wildtype mice. Furthermore, a few cross-sectional studies reported a positive association between vitamin D and testosterone.
Study design, size, duration: A single-center, randomized clinical trial with 307 infertile men randomized to receive placebo or a single dose of cholecalciferol 300,000 IU followed by 1400 IU + 500 mg of calcium daily for 150 days.
Participants/materials, setting, methods: Men included in the randomized clinical trial were initially referred to our andrological outpatient clinic due to infertility and subsequently invited to participate in the study. The primary endpoint was change in semen quality, while serum testosterone was a secondary endpoint.
Main results and the role of chance: Treatment of infertile men with high-dose vitamin D did not affect sex steroid levels. There was no difference in serum levels of testosterone, estradiol, LH or FSH between vitamin D and placebo treated men. However, a predefined subgroup analysis of men with vitamin D insufficiency and high PTH at baseline had significant higher free testosterone (Δ24%; p=0.027) and free testosterone/LH ratio (Δ34%; p=0.048) after 150 days vitamin D and calcium treatment compared with the placebo-treated men.
Limitations, reasons for caution: Our biggest limitations are the small number of men in the subgroupanalyses. Our findings should be verified in large clinical trials preferably in men with persistent vitamin D insufficiency characterized by low serum 25-OHD and high PTH.
Wider implications of the findings: Our findings suggest an increased awareness on vitamin D status in infertile men which may be important to avoid deterioration of their Leydig Cell function
Trial registration number: NCT01304927

P-032 Study on the specific piRNAs in human seminal plasma as a non-invasive diagnostic indicator before microsurgical testicular sperm extraction

X. Huang[1], **S. Xie**[1]

[1]*Center for Reproductive Medicine- Tongji Medical College- Huazhong University of Science and Technology- China, Department of Andrology, wuhan, China*

Study question: Clinical studies have not found an effective non-invasive test to accurately determine the presence of sperm in the testicles of non-obstructive azoospermia (NOA) patients
Summary answer: Except AZFa and AZFb microdeletion for a small group of NOA, no other reliable diagnostic technique has been widely accepted nowadays.
What is known already: piRNAs are a series of specific small molecule nucleic acid produced in the testis of different mammals. Previously, we compared piRNAs expression profiles in the testicles of NOA patients with successfully identified sperm and those without sperm by using second-generation sequencing. We identified and screened a variety of piRNAs with significant differences between the two groups.
Study design, size, duration: We randomly selected 120 NOA patients who were interested in micro-TESE and collected their ejaculated semen prior to microsurgery for piRNAs detection.
Participants/materials, setting, methods: According to our previous sequencing results, we screen out eight specific piRNAs. Before Micro-TESE performed for a total of 120 NOA cases, these piRNAs in human seminal plasm were detected by Fluorescent quantitative PCR. Each specimen was tested three times. Patients were classified into two groups (successful sperm retrieval [SSR] and unsuccessful sperm retrieval [USR]) after the operation of micro-TESE. Statistics were conducted according to the results of micro-TESE and seminal plasma piRNAs detection.
Main results and the role of chance: Three piRNAs DQ578561, DQ576926 and DQ577970 were found to have significantly higher expressions in the SSR group (51 cases) than the USR group (69 cases).
Limitations, reasons for caution: A relative small sample without pathological classification of testicular tissue in the patients with NOA.
Wider implications of the findings: piRNA DQ578561, DQ576926 and DQ577970 are expected to be new diagnostic indicators for non-invasive prediction of testicular residual sperm in NOA patients, which need further clinical verification with a larger sample size in multiple centers.
Trial registration number: No. 31671551

P-033 A new container with higher success rate for the cryopreservation of testicular tissues and motile spermatozoa from mice and testicular cancer patients

K. Nakata[1], **S. Wakayama**[2], **M. Iijima**[3], **T. Wakayama**[2], **H. Nakata**[3], **A. Mizogami**[3], **N. Yamashita**[4]

[1]*Yamashita shonan yume clinic, Reproductive Medicine Reserch Center, Kanagawa, Japan ;*
[2]*University of Yamanashi, Faculty of life and environmental sciences, Yamanashi, Japan ;*

[3]Kanazawa University, Integrative Cancer Therapy and Urology- Division of Cancer Medicine- Graduate School of medical Science, Kanazawa, Japan ;
[4]Yamashita Shonan Yume Clinic, Doctor division, Kanagawa, Japan

Study question: Is the new container effective for the freezing and thawing of the testicular tissues (TT) and motile spermatozoa?
Summary answer: The new container is effective for the cryopreservation of TT in order to preserve motile spermatozoa from mice and testicular cancer patients.
What is known already: Most men diagnosed with testicular tumors are between ages 15-40. Although the European Association of Urology recommends all testicular cancer patients are referred for semen analysis and cryopreservation prior to treatment, it was reported that 6-24% of testicular cancer patients were azoospermia, 50% were Oligozoospermia. Because testicular cancer patients have a low sperm count and may not be able to collect enough sperm for ART, TT banking is recommended. Commonly, straws or vials have been used for cryopreservation of TT. There are no studies referring to the comparison between different methods. But after thawing, motility of sperm is very low.
Study design, size, duration: Mouse TT was divided into nine groups to examine the effect of the combination of freezing speed (slow vs. rapid), freezing medium (Cellbanker1 vs. Fertiup), and freezing vessel (New container vs. Cryotube), and control (Fresh), repeatedly. That is, SCN, SFN, RCN, RFN, SCC, SFC, RCC, RFC and Control. TT from Testicular cancer patients was divided into two groups using slow freezing, with (Cellbanker1 vs, Sperm freezing) and the new container, that is SCN and SSN.
Participants/materials, setting, methods: Mouse and testicular cancer patient's TT were moved into the physiological saline solution and cut into 2-3 mm pieces with the scissors. Mice TT was frozen in 9 groups, and testicular cancer patient's TT was frozen in 2 groups. After thawing, we observed sperm motility, sperm membrane damage and Caspase-3 assay in mice and testicular cancer patients. Also *In vitro* and *in vivo* development after embryo transfer were assessed in mice.
Main results and the role of chance: The average number of mouse motile spermatozoa after thawing was examined. The motility rate was 25.7±13.6 % in SCN. PI positive rate was 20.0±10.0 in SCN. SCN was significantly higher than the other groups in motility and PI positive rate (P<0.05). The highest rate was obtained when TT was frozen using slow freezing, Cellbanker1 and New container[若山1] . After ICSI, the blastocyst rates were 54.9±13.6 % in SCN, 48.4±25.7 % in SFN, 40.8±16.2 % in SCC, 20.6±25.4 % in SFC and 64.2±10.8 % in Control, respectively. SCN was significantly higher than SFC (P<0.05). Additionally, we obtained fetuses 22.4% in SCN, 22.8% in SFN, 15.4% in SCC, 14.3% in SFC and 42.0% in Control. Five testicular cancer patient's tissues were frozen by SCN or SSN. After thawing, sperm motility rates of 5 patients were 18.9±13.2% (477/2681) in SCN and 29.6±13.2% (1415/4832) in SSN. SSN was significantly higher than SCN (*P*<0.05). PI positive sperm rates of 3 patients were 53.3±20.1% (293/460) in SCN and 29.6±12.5% (159/469) in SSN. SSN was significantly higher than SCN (*P*<0.02). In Caspase-3 assay of 3 patients, negative cell number was 127 in SCN, 255.5 in SSN and 1046.5 in control, respectively.
Limitations, reasons for caution: This is a basic study on a relatively small sample size with limited conditions. The confirmation using larger samples under various conditions may be required. Furthermore, although healthy mouse offspring was obtained, we need to check the safety of using TT frozen-thawed sperm in ART.
Wider implications of the findings: The findings of this study indicate that the new container allows checking inside the seminiferous tubule using microscope. Because it's made of polydimethylsiloxane and the bottom is transparent and very thin. It is also useful for a small number of spermatozoa (K. Nakata, et al. 2019) and testicular tissues cryopreservation.
Trial registration number: No

P-034 Impact of tobacco smoking on the expression level of H2BFWT, TNP1, TNP2, PRM1 and PRM2 genes in spermatozoa

H. Amor[1], M.E. Hammadeh[1]

[1]University of Saarland, Obstetrics and Gynecology, Homburg/Saar, Germany

Study question: Can Tobacco smoke alter the expression level of H2BFWT, TNP1, TNP2, PRM1, and PRM2 genes?
Summary answer: Can Tobacco smoke alter the expression level of H2BFWT, TNP1, TNP2, PRM1 and PRM2 genes in spermatozoa?
What is known already: Various studies were focusing on the mechanisms by which the environmental and lifestyle factors especially smoking influence on the sperm genome and epigenome.

Testis-specific histones like H2BFWT, transition proteins TP1 and TP2 and protamines P1 and P2 are the main nuclear proteins that have a crucial role during the protamination of sperm genome during spermiogenesis. Protamination leads to the elimination of histones carrying epigenetic signals. Thus, protamination take part in the epigenetic regulation of the spermatozoa and any factor changing protamination may be considered as an epigenetic signal like DNA methylation and histone modification influencing the transcription regulation after fertilization.
Study design, size, duration: A prospective controlled trial carried between August 2016 and April 2018 at the Department of Obstetrics and Gynecology, University of Saarland, Germany. 167 semen samples were included in this study and divided into 54 non-smokers (G1) and 113 heavy-smokers (G2).
Participants/materials, setting, methods: After semen purification, total RNA was isolated using Isolate II DNA/RNA/Protein kit then concentration and purity were checked with Nanodrop spectrophotometer ND-2000c. RT-qPCR technique was used for the quantification of the expression level of the five studied genes using miScript reverse transcription and QuantiTect SYBR Green PCR Kits. Protamine deficiency (CMA3+) was assessed by Chromomycine CMA3 staining and sperm DNA fragmentation (sDF) by TUNEL assay.
Main results and the role of chance: In G1, the sperm count (88.09± 63.42 mill/ml), progressive motility (27.31±21.78%), normal morphology (10.87±12.11%), were significantly higher (p<0.01) than G2 (62.17± 51.68 mill/ml; 14.86±10.95%; 4.01±2.88%, respectively).

Whereas, CMA3+ (23.50±14.70%) and sDF (17.41±14.59%) in G1 were significantly lower (p<0.01) than G2 (33.58±21.34%; 27.55±20.01%, respectively).

Protamine mRNA ratio was significantly higher in G1 in comparison to G2 (0.11 ± 0.84 vs. 0.60 ± 1.08; p=0.001). Unlike G1, the protamine mRNA ratio significantly correlates with CMA3+ (r=0.413, p=0.0001) and sDF (r=0.302, p=0.003) in G2.

Moreover, the relative amounts of each studied gene mRNA (mean delta ct) were differentially expressed between G1 and G2 and this difference was highly significant (p<0.01). Besides, H2BFWT, TNP1, TNP2, PRM1, and PRM2 genes were down-regulated in spermatozoa of G2 compared to G1 (Fold change <0.5).
Limitations, reasons for caution: The size number of the sample.
Wider implications of the findings: Studied genes are expressed in a well-organized chronological manner and any alterations from an internal or external factor like smoking my altered this mechanism and thus altered spermiogenesis and sperm function.
Trial registration number: not applicable

P-035 Sperm DNA integrity and human papillomavirus (HPV) infections: a controversy that could be resolved by a new molecular approach

R. Schillaci[1], T. Notari[2], G. Capra[3], M.A. Ragusa[4], M.C. Roccheri[4], A. Perino[3], A. Ferrigno[4], L. Bosco[4]

[1]Centro Interaziendale di Procreazione Medicalmente Assistita- Ospedale "V. Cervello"- Università di Palermo- Italy, Department of Sciences for Health Promotion and Mother-Child Care 'G. D'Alessandro' PROSAMI, Palermo, Italy ;
[2]Check Up Research Unit - Polydiagnostic and Day Surgery Centre - Salerno, Check Up Research Unit - Polydiagnostic and Day Surgery Centre, Salerno, Italy ;
[3]University of Palermo, Department of Sciences for Health Promotion and Mother-Child Care 'G. D'Alessandro' PROSAMI, Palermo, Italy ;
[4]University of Palermo, Department of Biological- Chemical- and Pharmaceutical Sciences and Technologies STEBICEF, Palermo, Italy

Study question: The aim was to determine if HPVs affect spermatozoa DNA integrity. To resolve the discrepancy regarding the association between HPV infections and sperm DNA damage.

Summary answer: To elucidate if HPV impairs DNA integrity, we suggest to investigate HPV DNA positivity in spermatozoa by a differential lysis procedure before TUNEL assay.

What is known already: HPV can influence human fertility, in fact it has been demonstrated that infected couples undergoing assisted reproduction techniques showed an increased risk of pregnancy loss compared with non-infected counterparts.

Recent studies revealed that DNA Fragmentation Index (DFI) was not different in HPV-infected and in non-infected semen samples. Other evidences reported statistically significant differences. Therefore, it remains controversial whether HPV infection in semen is associated to DNA damage.

Moreover, it is known that HPV DNA can be found in the different semen components, then it is necessary to investigate if patients defined as HPV-positive have viral DNA contained in the spermatozoa.

Study design, size, duration: From April 2015 to October 2019, a total of 105 patients: 56 HPV positive and 49 HPV negative, male partners of women with High Grade Squamous Intraepithelial Lesions (HSILs) positive for high risk (HR) HPVs were enrolled in an observational study.

Participants/materials, setting, methods: Patients who met the following inclusion criteria were enrolled: age between 18 and 50 years; partner positive for HR-HPV types; sperm concentration after swim up procedure that exceeded 1 million/ml. Exclusion criteria were: varicocele; cryptorchidism; other genital infections; chemo/radio therapy. Seminal parameters were evaluated according to 2010 World Health Organization guidelines and sperms were then separated by swim-up technique. HPV-DNA was detected and genotyped by reverse hybridization. DFI was evaluated by TUNEL assay.

Main results and the role of chance: As we have previously demonstrated, no statistically significant effect of HPV infection on sperm parameters was observed when we compared HPV-positive total semen samples with respect to HPV-negative counterparts. However samples showing higher viral DNA ratio had lower percentage of progressively motile sperms.

In our experience, DFI values are not different in HPV positive and in negative samples (17% *vs* 20% respectively).

This result could be explained by the following considerations. Our previous data showed that HPV DNA can be identified in every fraction of semen, by differential lysis procedure: spermatozoa, somatic cells and seminal plasma. Different samples can contain HPV DNA in different fractions and a sample could result HPV-positive also in absence of viral DNA into spermatozoa. Therefore, it is necessary to determine which semen fraction contains viral DNA in order to classify samples as positive or negative with respect to spermatozoa infection before to perform TUNEL assay, since DFI is evaluated exclusively into spermatozoa.

Limitations, reasons for caution: Low number of samples because of the difficulty to find male partners agreeable to the enrollment in the study.

Wider implications of the findings: Semen parameters and DFI may have no significant differences with respect to HPV positivity detected by conventional procedures. Therefore, it is advisable to perform the differential lysis procedure in HPV-positive patients to verify in which fraction the virus is located and as a consequence its potential effect on male fertility.

Trial registration number: Not applicable

P-036 Will the use of testicular spermatozoa optimise reproductive outcomes?

R. Mani Raghupathy[1], K.C. Mantravadi[2], D. Rao Gedela[3]

[1]Oasis Centre of Reproductive Medicine, Clinical Embryology, Hyderabad, India ; [2]Oasis Centre for Reproductive Medicine, Clinical Embryology, Hyderabad, India ; [3]Oasis Centre for Reproductive Medicine, Reproductive Medicine, Hyderabad, India

Study question: Will TESA (Testicular Sperm Aspiration) be a beneficial procedure for couples with raised DFI in the male partner.

Summary answer: Testicular spermatozoa obtained surgically from individuals with raised DFI, will benefit the couple in achieving optimal reproductive outcomes

What is known already: Raised DFI (DNA Fragmentation Index) is one of the major causes of male infertility and is known to affect fertilisation, embryo quality, implantation, miscarriage & pregnancy rates after assisted reproduction. ROS is known to attack and damage the spermatozoal DNA happens as spermatozoa travels through the epididymis. And hence it is widely understood and accepted that testicular spermatozoa have a relatively lesser degree of DNA fragmentation. Surgical sperm retrieval techniques have become more refined over the years and now yield good quality testicular spermatozoa

Study design, size, duration: Data was collected retrospectively from 2015, till 2018. SCSA (Sperm Chromatin Structure Assay) was done to assess the DNA fragmentation Index (DFI) of male partner of all couples who had one failed IVF cycle. Those with DFI>30 were identified. Couple with female partners age>35 years were excluded. The Fertilisation, Blastocyst, Implantation, Clinical Pregnancy, Miscarriage, Live Birth rates, along with Perinatal and neonatal outcomes of the subjects were then compared.

Participants/materials, setting, methods: 605 subjects were included in the study based on the pre-requisites Those, who discontinued treatment, or used Donor sperms for ICSI, were excluded. Couple who chose ICSI (Intra Cytoplasmic Sperm Injection) with ejaculate (n=14) were included in the control group, and the study group included all who chose ICSI with TESA obtained spermatozoa (n=37). TESA was done after taking all aseptic precautions and following the defined procedural Standard Operating Protocol.

Main results and the role of chance: The embryo parameters, Fertilisation Rate (TESA = 96.59%; Non-TESA = 89.88%) & Blastocyst Rate (TESA = 33.65%; Non-TESA = 40.95%) in both groups were comparable. The implantation rates were higher for the study group (TESA= 60.0%; Non-TESA = 23.1%) in the control group. Pregnancy induced hypertension and IUGR were not observed in both the groups. The Clinical pregnancy (TESA = 63.2%; Non-TESA = 28.6%) and Live birth rates (TESA = 42.1%; Non-TESA = 28.6%) were better in the study group. 4 subjects in the TESA group have ongoing pregnancy There were no adverse perinatal and neonatal outcomes in the study group. Though Blastocyst rates seemed better in the Non-TESA group, reproductive outcomes were better in the TESA group. And also, the perinatal and neonatal outcomes were confirming the safety of using TESA obtained spermatozoa for ICSI in couples with raised DFI.

Hence, based on the data obtained from the study, it is safe to assume that the use of testicular spermatozoa for raised DFI seems to be a safe and beneficial intervention.

Limitations, reasons for caution: The major limitations of this study is that this is a retrospective study with a small sample size, along with the fact that TESA is an invasive intervention

Wider implications of the findings: Male partners with raised DFI could be offered TESA as a treatment option, benefiting couples with optimal reproductive outcomes.

Trial registration number: Not Applicable

P-037 Body mass index and age correlate with antioxidant supplementation effects on sperm quality: Post-hoc analyses from a double-blind placebo-controlled trial

G.M. Busetto[1], F. Del Giudice[1], A. Virmani[2], E. De Berardinis[1], A. Agarwal[3]

[1]Sapienza University Policlinico Umberto I, Urology, Roma, Italy ; [2]Alfasigma HealthScience, Nutraceuticals, Utrecht, The Netherlands ; [3]American Center for Reproductive Medicine, Andrology Center, Cleveland, U.S.A.

Study question: Is there any correlation between age and body mass index (BMI) with the efficiency of medical therapy for infertile men?

Summary answer: Medical therapy with compounds is more effective in subjects with varicocele younger than 35 years and with BMI <25.

What is known already: The decline of male fertility is an emerging problem and causes for these changes are lifestyle factors and global changes in our eating habits with increasing evidence of obesity. Spermatozoa are vulnerable to lack of energy and oxidative stress as a result of elevated levels of reactive oxygen species. Therefore, it is essential that appropriate nutrients are available during maturation. At present, no studies investigated the correlation of age and body mass index (BMI) with the efficiency of a medical therapy in infertile men.

Study design, size, duration: The present analysis stems from the extensive database that has been created to determine the effects of antioxidant supplementation on semen quality. This database includes 104 infertile patients with oligo- and/or astheno- and/or teratozoospermia with an average age of 32.5 years, enrolled in a randomized, double-blind, placebo-controlled trial. Included

are 52 patients with grade I-III varicoceles and 52 patients without varicocele that were divided into supplementation or placebo groups.
Participants/materials, setting, methods: In accordance with the randomization schedule, subjects received 2 packets of either supplement or placebo daily for 6 months. Semen parameters were evaluated in a standard semen analysis at the beginning of the treatment (V1) and after completing 6 months of therapy (V2). Pregnancy rate was included as a secondary outcome. The present post-hoc analyses were carried out on the samples as categorized by age/BMI and presence/absence of varicocele.
Main results and the role of chance: One of the primary aims of this study was to correlate the results of the semen analysis with BMI and age. In particular, we wanted to see if aging and obesity status would decrease efficacy of the supplementary antioxidant treatment on main sperm parameters (see Tables 1-5). For BMI, a significant difference was observed in the BMI <25 group with varicocele for total sperm count (p=0.0272) and progressive motility (p=0.0159). No statistical significance was observed in the combined classes. The results were partially confirmed by carrying out the Chi-Square test on the data arranged as "Responder/Non Responder". As for the total sperm count, in both the BMI <25 and the combined varicocele group (i.e. BMI <25 and age <35) a statistical difference was observed (p=0.0066 and p=0.0078 respectively). These post-hoc analyses suggest that the nutritional supplement seems to be more effective in subjects younger than 35 years with a BMI below 25.

Looking at other parameters, patients treated with compounds obtained a statistically significant improve of sperm parameters for the following items: total count, progressive and total motility, morphology.

As a secondary outcome, 12 pregnancies occurred during the follow-up time: 10 in the supplementation group and 2 in the placebo group.
Limitations, reasons for caution: Even as a double-blind placebo-controlled study with very strict inclusion and exclusion criteria, we did not include sperm DNA fragmentation. Also, an oxidative stress measure such as ORP was not included. There also may be other factors besides aging and obesity involved, including lifestyle, associated disease and fat distribution.
Wider implications of the findings: In addition to earlier findings regarding improved sperm parameters in supplemented patients, these post-hoc analyses suggest that antioxidant supplementation seems to be more effective on improving sperm parameters in subjects aged less than 35 years old and with BMI below 25.
Trial registration number: NCT04177667

P-038 New insights into the physiopathology of teratozoospermia and its association with sperm DNA defects, apoptotic alterations and oxidative stress

O. Ammar[1], M. Mehdi[2], A. Sallem[2], M. Muratori[3]

[1]Faculty of Medicine University of Monastir-, Laboratory of Histology Embryology and Cytogenetic LR 18-ES 40-, Monastir, Tunisia ;
[2]Faculty of Medicine- University of Monastir, Laboratory of Histology Embryology and Cytogenetics LR 40 ES 18, Monastir, Tunisia ;
[3]Center of Excellence DeNothe, Department of Experimental and Clinical Biomedical Sciences "Mario Serio"- Unit of Sexual Medicine and Andrology, Florence, Italy

Study question: This study set out to determine the level of sperm nuclear DNA damage in patients with isolated poymorphic teratozoospermia and examining its relationship with oxidative stress and apoptosis.
Summary answer: Decreased seminal antioxidant profile may be an important factor involved in the mechanism of sperm cell death-mediated DNA breaks in teratozoospermic semen.
What is known already: Sperm morphological defects is associated with apoptosis.
Study design, size, duration: A total of 89 patients was divided into two groups, men with isolated teratozoospermia (n = 69) and men with normal semen parameters (n = 20) as controls.
Participants/materials, setting, methods: Sperm DNA breaks were evaluated by using acridine orange staining. The proportion of viable spermatozoa with mitochondrial transmembrane depolarization was detected by fluorescence microscopy through the use of MitoPT-JC-1 staining method. Bivariate Annexin V/ 6-CFDA analysis was then carried out to measure the percentage of both viable and dead spermatozoa with phosphatidylserine (PS) externalization. Seminal antioxidant profile (reduced Glutathione (GSHr); Oxidized Glutathione (GSSG); Glutathione-S-transferase (GST)), and total protein sulfhydryl (P-SH) concentrations were measured spectrophotometrically.
Main results and the role of chance: Patient with isolated teratozoospermia, when compared to fertile donors, showed significantly increased level of single sperm DNA breaks, and higher proportions of spermatozoa with phosphatidylserine externalization and mitochondrial depolarisation. Among the different studied oxidative stress seminal parameters, the rates of seminal GSHr, GST and P-SH were significantly decreased in the patient group. However, the seminal levels of GSSG and GST have decreased, but only GST didn't showed a significant difference. Interestingly, significant relationships were found between the studied apoptotic markers and the rate of atypical sperm forms with the incidences of head abnormalities. Furthermore, positive inter-correlations were found between sperm DNA defects, impaired seminal antioxidant profile and the sperm apoptotic markers.
Limitations, reasons for caution: Further combined analysis of oxidative stress, apoptotic markers and nuclear defects should provide complementary measurements for the evaluation of sperm quality and could contribute to provide adequate reproductive and genetic counselling for hypofertile patients with isolated polymorphic teratozoospermia.
Wider implications of the findings: Sperm DNA defects as well as apoptosis and seminal oxidative stress are interlinked in the context of teratozoospermia, and constitute a unified pathogenic molecular mechanism
Trial registration number: not applicable

P-039 In spermatozoa collected after pellet swim up, when total dna fragmentation is higher than 15%, the normal morphologically spermatozoa population shows an increased dna damage.

G. Ruvolo[1], A. Ferrigno[2], M.C. Roccheri[2], E. Cittadini[1], A. Pane[1], C. Simonaro[1], B. Ermini[3], I. Camera[3], A.M. Bruccoleri[1], M. Manno[1], A. Lo bue[1], L. Bosco[2]

[1]Centro di Biologia della Riproduzione, Centro di Biologia della Riproduzione, Palermo, Italy ;
[2]University of Palermo- Palermo- Italy, Department of Biological- Chemical- and Pharmaceutical Sciences and Technologies STEBICEF-, Palermo, Italy ;
[3]CIPA- Centro Italiano di Procreazione Assistita, CIPA- Centro Italiano di Procreazione Assistita, Roma, Italy

Study question: We investigated the DNA Fragmentation Index (DFI) in motile normal morphologically spermatozoa comparing samples with total DFI < 15% Vs ≥ 15% collected after pellet swim up
Summary answer: In the case of DFI ≥15% the percentage of normal morphologically spermatozoa with fragmented DNA is significantly higher than the population with DFI < 15%
What is known already: Intracytoplasmic sperm injection (ICSI) is widely used in the treatment of male infertility. Only morphologically normal spermatozoa are mainly used by embryologists to fertilize an oocyte. Different papers have reported that spermatozoa with apparently normal morphology may have DNA fragmentation. These evaluations suggest that it is possible that normal-shaped spermatozoa but with DNA fragmentation could be easily selected to fertilize oocytes during ICSI. It is known that the presence of an increased proportion of normal spermatozoa with damaged DNA is negatively associated with embryo quality affecting both pregnancy and implantation outcomes after ICSI.
Study design, size, duration: We designed an observational study on 70 male patients. We speculated that the examination of DNA integrity in motile and morphologically normal sperm, collected after pellet swim up, could provide useful information concerning sperm competence, rather than the DFI evaluation in the raw seminal sample. We analyzed data from January 2019 to December 2019. The aim is to demonstrate that DFI in normal morphologically spermatozoa, could be indicated as predictive parameter of ICSI success.
Participants/materials, setting, methods: DFI and traditional semen parameters (WHO, 2010), were evaluated in all patients. DFI was calculated using *in situ* TUNEL assay in at least 250 spermatozoa. By means of NIS-Elements BR 3.10 image analyzer software (Nikon) using images of the same field (light, fluorescence and "merged") it was possible to evaluate sperm morphology associated with DNA fragmentation. Data were analyzed using the Kruskal-Wallis

test, a non-parametric ANOVA, confirmed by restrictive Bonferroni correction using the Dunn's test.

Main results and the role of chance: In this observational study we included 70 oligoasthenospermic patients undergoing ICSI. The patients were classified in 2 groups according to the sperm DFI: Group A (n=35) included those who had a DFI < 15% in the population of sperm collected after swim up. In group B (n= 35) patients with a DFI ≥ 15%. We did not find any statistical difference between the two groups in the traditional sperm parameters like density, motility and morphology.

We observed that, in Group A, the average value of the total of sperm DFI was 9.32% while in Group B was 24.71 % (p< 0.0001). When the analysis was restricted only to spermatozoa with normal morphology, it was observed that among patients of Group B the DFI value was 13.6%, while in A Group the average DFI value was 2.2%, with a strong statistical difference (p<0.0001). DFI calculated on motile, normal morphologically spermatozoa can provide an important information on the probability and risk of injecting, during ICSI procedure, a sperm with normal morphology but with fragmented DNA. This risk is higher if the sperm population collected after pellet swim up has a DFI higher than 15%.

Limitations, reasons for caution: This type of analysis only provides a prediction to select a sperm with fragmented DNA, but does not allow the selection of single spermatozoa with intact DNA to be used for ICSI. Further studies are needed to correlate these data with the clinical outcome.

Wider implications of the findings: Our results suggest that the evaluation of DFI in morphologically motile normal sperm selected after pellet swim up appears to be a more accurate strategy to evaluate the sperm competence, with the aim to improve the ICSI outcomes, than the traditional evaluation of sperm DFI in the whole seminal sample.

Trial registration number: not applicable

P-040 The impact of motility, morphology and presence of testicular spermatozoa on fertilization, embryo development and live birth rates, in fresh and frozen testicular samples

C. Oraiopoulou[1], A. Vorniotaki[1], E. Taki[1], A. Papatheodorou[1], M. Moissidou[1], N. Christoforidis[1], A. Chatziparasidou[1]

[1]Embryolab IVF Clinic, Embryolab, Thessaloniki, Greece

Study question: Does cryopreservation or the quality parameters of testicular spermatozoa in fresh or frozen samples have an impact on fertilization rate, embryo development and live birth rate?

Summary answer: Although live birth rate (LBR) is not directly associated to any parameters examined, morphology and motility of testicular spermatozoa influence the number of available embryos.

What is known already: Almost 5% of couples undergoing IVF treatments are confronted with azoospermia and are counseled towards TESE-ICSI cycles. At the same time, it has been reported that there is no influence of the use of cryopreserved testicular sperm in fertilization rate and live birth rate and in the presence of motile spermatozoa, high embryo quality and pregnancy rates are expected. Motility of the spermatozoa during the ICSI procedure has been associated to live birth, while other studies claim that motility of either fresh or frozen/warmed testicular spermatozoa is the only parameter associated to ongoing pregnancy.

Study design, size, duration: A retrospective cohort study between 01/2014 and 12/2017 was performed in Embryolab IVF Clinic, Greece, including 108 TESE-ICSI treatment cycles. Logistic regression analysis was used to explore the influence of variables (fresh-frozen/warmed testicular tissue, presence/motility/morphology of testicular spermatozoa) in fertilization rate, embryonic development and LBR. Women above 38 years old, frozen oocyte cycles, PGT cycles and couples with abnormal karyotypes were excluded from the study.

Participants/materials, setting, methods: Morphology, presence and motility were graded as: good/motile(1 grade), average/twitcher(2 grades), low/immotile(3 grades) and the sum of grades represented the total quality score for the testicular spermatozoa used for ICSI. Group A included cases with up to total grade 4, while Group B included cases with total grade 5 or higher. Embryo quality was evaluated up to day 3 (good quality: more than 5 blastomeres, less than 20% fragmentation). LBR was calculated per first transfer.

Main results and the role of chance: Fertilization rate was comparable (p>0.05) among fresh and frozen samples for both group A (fresh: n=24, 67%fertilization rate / frozen: n=33, 62% fertilization rate) and group B(fresh: n=23, 47%fertilization rate / frozen: n=38, 43% fertilization rate), with group A spermatozoa (n=27, 64%fertilization rate) performing significantly better compared to group B spermatozoa (n=91, 51%fertilization rate), p<0.05.

Group A spermatozoa produced significantly more good day 3 embryos compared to Group B (p<0.05), in particular 1,56 additional good quality embryos.

Interestingly, fresh Group B spermatozoa performed better than frozen Group B spermatozoa, resulting in significantly more good quality embryos on day 3 (p<0.05).

Furthermore, there was a significant decrease in good quality day 3 embryos, if both morphology and motility were graded as low/immotile (0.75 and 0.45 less good quality embryos respectively, p<0.05).

Although there was a trend for higher cycle cancellation rate in group B comparing to Group A, either fresh or frozen, the difference was not statistically significant.

Overall, although LBR was not affected by any parameter examined, the number of good quality embryos available for transfer was affected by both the quality of testicular spermatozoa used for ICSI and cryopreservation in low quality samples.

Limitations, reasons for caution: The evaluation of "presence" and "morphology" as good/average/bad could have a subjective complexion. However, this variation is eliminated through the grouping of samples.

Accumulative LBR was not calculated, as LBR was based only on the first transfer.

Wider implications of the findings: Since there is cryopreserved testicular tissue of good quality, there is no added value in proceeding to another surgery. However, in low quality samples, the use of fresh testicular spermatozoa could alter the final outcome, since cryopreservation affects the number of available good quality embryos.

Trial registration number: Not applicable

P-041 The effect of prolonged incubation of sperm at testis temperature (35°C) versus room temperature (26°C) on semen parameters

M. Mehrafza[1], C. Ostadian[2], A. Hosseini[3], A. Eftekhari[3], M. Asgharnia[1], S. Aghajani[3], H. Vahabzadeh[3], M. Gholami[3], A. Raoufi[4], S. Samadnia[4], E. Hosseinzadeh[4]

[1]Mehr Fertility Research Center- Guilan University of Medical Sciences- Rasht- Iran, Gynaecology, Rasht, Iran ;

[2]Mehr Fertility Research Center- Guilan University of Medical Sciences- Rasht- Iran, Andrology, Rasht, Iran ;

[3]Mehr Fertility Research Center- Guilan University of Medical Sciences- Rasht- Iran, Embryology, Rasht, Iran ;

[4]Mehr Fertility Research Center- Guilan University of Medical Sciences- Rasht- Iran, Research, Rasht, Iran

Study question: Does prolonged incubation of sperm at 35° C versus room temperature (26 ° C) affect semen parameters and DNA fragmentation index (DFI)?

Summary answer: The concentration and motility of spermatozoa were significantly higher in room temperature than 35°C. However, Temperature had no effect on DFI after 24 h.

What is known already: Currently, cryopreservation is used routinely for prolonged storage of sperm even for one day, which, despite its high cost, can affect the quality of sperm samples. If long-term incubation of sperm in the laboratory environment is possible without affecting its quality, it will be possible to manage the patient's treatment with higher quality and with greater choice**.**

Study design, size, duration: In the present experimental study, sperm samples were collected from 40 participants referred to Mehr Medical Institute, Rasht, Iran, from September 2019 to December 2019.

Participants/materials, setting, methods: Each semen sample was divided into two equal parts and was subjected to swim-up procedures. One group was incubated at 35°C and the other at room temperature, in the darkness. Both groups were evaluated for number, motility (Grade A and B) and morphology at 45 min, 24 h and 48 h intervals. Statistical analysis was

performed using repeated measure analysis of variance (ANOVA) and student´s t-test.
Main results and the role of chance: Sperm concentration (P = 0.007) and motility (P <0.001) was significantly higher in room temperature than 35°C, at three intervals of 45 minutes, 24 hours and 48 hours. However, the mean values of normal morphology spermatozoa was not significantly different between the two groups at three intervals of 45 minutes, 24 hours and 48 hours (P = 0.08). Also, there was no significant difference in DFI at 26 and 35°C after 24 h (P = 0.2).
Limitations, reasons for caution: At the time of the abstract preparation, 20 samples had not been read for DFI.
Wider implications of the findings: Sperm morphology was significantly reduced following prolonged incubation.
Trial registration number: not applicable

P-042 Clinical outcomes in patients with congenital bilateral absence of the vas deferens (CBAVD) undergoing testicular sperm extraction-intracytoplasmic sperm injection (TESE-ICSI)

T. Ishikawa[1], S. Mizuta[1], K. Yamaguchi[1], H. Matsubayashi[1], T. Takeuchi[2], K. Kitaya[1]

[1]Reproduction Clinic Osaka, Reproductive Medicine, Osaka, Japan ; [2]Reproduction Clinic Tokyo, Reproductive Medicine, Tokyo, Japan

Study question: What is the fertilization rate, embryonic development, and clinical outcome for TESE-ICSI using testicular spermatozoa among obstructive azoospermia (OA) including CBAVD couples?
Summary answer: Clinical pregnancy rate (CPR) per embryo transfer (ET) by testicular sperm did not differ significantly between CBAVD and other OA patients.
What is known already: OA is caused by congenital including bilateral absence of the vas deferens (obstructed in the long term) or acquired (vasectomy, inguinal hernioplasty in childhood, etc.). It is known that long-term obstruction is related to a defect of spermatogenesis, however, clinical outcomes after ICSI with testicular sperm in the etiology OA, classified as congenital or acquired causes have hardly been investigated.
Study design, size, duration: We performed a retrospective study based on two reproduction centers in Japan and evaluated 206 NOA patinets including 50 cases with CBAVD performed in our clinic between September 2013 and December 2019. In addition, a total of 108 TESE-ICSI cycle with 47 couples for CBAVD and 293 TESE-ICSI cycles with 138 couples for other obstruction were performed. Sperm retrieval rate was 100% and TESE-ICSI was performed with only motile spermatozoa.
Participants/materials, setting, methods: The diagnosis of CBAVD is based on normal-size testes (>16mL), normal FSH and nonpalpable vas deferens. The diagnosis of OA also required confirmation of normal spermatogenesis by testicular biopsy. TESE-ICSI cycles were evaluated in embryonic development rates and CPR. Additionally, paternal and maternal age, numbers of oocytes retrieved, baseline FSH, LH and E2 levels of women, and FSH, LH and Testosterone of men were compared between the two groups.
Main results and the role of chance: The wives age at ICSI was 33.9±4.4 years for CBAVD and 35.9±5.1 years for other obstruction. The sperm retrieval rate with TESE was 100%, in which motile spermatozoa were retrieved and used for ICSI for all patients. Two pronuclei (2PN), blastocysts development, and good-quality blastocysts rates were 57.2%, 44.8%, and 19.6% in CBAVD and 64.0%, 53.3%, and 23.0% in other obstruction, respectively. 2PN and blastocysts development rate in CBAVD was significantly lower than in other obstruction (P<0.001). However, no significant difference was found in good-quality blastocysts rates. Embryo transfer was performed 44 couples with CBAVD patients undergoing TESE-ICSI which were divided into clinical pregnancy positive (n=37) and negative (n=7) groups. There was no difference for paternal and maternal age, numbers of oocytes retrieved, baseline FSH, LH and E2 levels of women, and FSH, LH and Testosterone of men. CPR per ET cycle and per couple were similar in CBAVD (40.2% and 84.1%, respectively) and in other obstructive (41.3% and 79.4%, respectively).
Limitations, reasons for caution: There was a lack of accurate term of obstruction with respect to not CBAVD OA patients. Some other obstructive patients could be congenital but not acquired. Additionally, the safety and screening for congenital malformations among these children has not been fully investigated.
Wider implications of the findings: Long-term obstruction is not related to a sperm retrieval by conventional TESE and clinical pregnancy, however, embryonic development were not similar as the other OAs. In CBAVD couples, the selection of motile sperm from testicular tissue could be a critical key to succeed and rationale for good embryonic development.
Trial registration number: not applicable

P-043 Sperm count affects cumulative live birth rate of assisted reproduction cycles in relation to ovarian response

C. Zaca'[1], G. Coticchio[1], N. Tarozzi[1], M. Nadalini[1], C. Lagalla[1], A. Garolla[2], A. Borini[1]

[1]9.Baby, Family and Fertility Center, Bologna, Italy ; [2]University of Padova, Department of medicine - Unit of andrology and reproductive medicine, Padova, Italy

Study question: Does sperm quality, as assessed by prewash total sperm count (TSC), affect cumulative success rates in assisted reproduction cycles?
Summary answer: Sperm quality impacts on the efficacy of IVF treatments primarily in cases of reduced ovarian response, but not in normal or high responders.
What is known already: The clinical outcome of treatments is influenced by numerous intrinsic and extrinsic factors. The female gamete is recognised as the single most important factor affecting the ability of the preimplantation embryo to implant and develop. For this reason, oocyte legacy has overshadowed the sperm role in embryogenesis. Regardless, TSC has been described as being highly predictive of male health in general and reproduction in particular. Its relative importance in determining the efficacy of IVF treatments remains uncertain and therefore demands thorough assessment, especially in the light of possible interactions between the male and female gametes
Study design, size, duration: Reported data concern a retrospective cohort study carried out between January 2009 to December 2013 involving 765 couples undergoing complete ICSI cycles, i.e. whose all embryos were transferred or disposed. Couples were characterised by male infertility, while female age was less than 36 years to minimise the well-documented maternal age effect on treatment outcome. Couples with a combination of female and male infertility factors were excluded.
Participants/materials, setting, methods: The cohort was grouped according to male partner's TSC into five groups, according to values discussed in the World Health Organization guidelines: **A)**< $0.1x10^6$ (189/765 cycles,24.7%); **B)**$0.1x10^6$ to $1x10^6$(144/756 cycles,18.8%); **C)**$1x10^6$ to $5x10^6$(150/756 cycles,19.6%); **D)**$5x10^6$ up to $10x10^6$ (103/756 cycles,13,5%); **E)**$10x10^6$ to $39x10^6$ TSC(179/756 cycles,23,4%).Groups were also analysed according to the number of oocytes retrieved. Mean age and number of oocyte was comparable among groups.Preimplantation genetic testing treatments and cycles in which spermatozoa were surgically recovered were excluded.
Main results and the role of chance: Mean age and number of retrieved oocyte was comparable among groups. The mean number of embryos across the different TSC groups was also similar (3.0±1.6, 2.8±1.7, 3.4±2.2, 3.1±1.8 and 3.2±1.9, respectively; P=0.066). Cumulative live birth rates (CLBR) were progressively higher with increasing TSC, reaching a plateau in groups with higher TSC (22.2%, 27.1%, 34.7%, 36.9%, 36.9%, respectively; P=0.01). On the contrary, miscarriage rates were comparable ranging from 14.7% to 25% (P=0.5). Furthermore, cumulative outcome rates were comparatively assessed in individual TSC groups in relation to different oocyte yield (1 to 5; 6 to 10, >10). As expected, in the most severe TSC condition (≤$0.1x10^6$) CLBR were progressively higher as the number of retrieved oocytes increased (5.3%, 21.5% and 30.2%, respectively; P=0.008). Similar trends were observed in the outcome of groups with TSC values of >$0.1x10^6$ to $1x10^6$ and >$1x10^6$ to $5x10^6$. On the contrary, in groups characterized by TSC of >$5x10^6$ to $10x10^6$ and >$10x10^6$ to $39x10^6$, CPR and LBR did not increase as a function of the number of retrieved oocytes.
Limitations, reasons for caution: The study design is retrospective and requires further refinement to control for factors that may impact clinical outcome.
Wider implications of the findings: This study highlights the general importance of sperm TSC for the efficacy of assisted reproduction treatments. The consequences of sperm quality on clinical outcome emerge decisively in case of reduced ovarian response, suggesting a relationship between ovarian

response and oocyte ability to compensate for paternal-derived deficiencies.
Trial registration number: Not applicable

P-044 Effect of paternal age on outcomes in ART cycles: A systematic review and meta-analysis

G. Morris[1], D. Mavrelos[1,2], E. Theodorou[1], M. Campbell-Forde[1], D. Cansfield[1], E. Yasmin[1,2], P. Sangster[1,2], W. Saab[1], P. Serhal[1], S. Seshadri[1]

[1]Centre for Reproductive and Genetic Health, Medical, London, United Kingdom ; [2]University College London Hospitals NHS Foundation Trust, Reproductive Medicine Unit, London, United Kingdom

Study question: Could paternal age have an independent effect on the clinical outcomes of ART cycles?
Summary answer: Autologous oocytes studies suggest increased male age reduces live birth and increases miscarriage. Donor oocytes studies found no impact of male age on ART outcome.
What is known already: There is no effect of paternal age on clinical outcomes in donor oocyte studies. However, studies where autologous oocytes were used, have demonstrated conflicting results with some suggesting that increased male age has a deleterious effect.
Study design, size, duration: A systematic review and meta-analysis of ten autologous oocyte cohort studies (including 11004 cycles) and 11 donor oocyte cohort studies (including 10338 cycles).
Participants/materials, setting, methods: The Cochrane Central Citation Index Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, NHS evidence and conference abstracts were searched for randomized controlled trials (RCT) and observational studies (OBS) that addressed the impact of paternal age on ART published up to December 2018. We searched reference lists of relevant articles and hand-searched relevant conference proceedings/abstracts. Data for women aged 39 years or under were extracted and analysed.
Main results and the role of chance: Clinical pregnancy rates were found to be statistically higher when the paternal age was under 40 years in autologous oocyte studies (OR 1.68, [1.24 – 2.28]; p = 0.0009) however there was no difference in clinical pregnancy in the same age category when donor oocyte studies were analysed (OR 0.95, [0.84 – 1.07]; p = 0.41). Livebirth rate (LBR) was reported in three autologous oocyte studies (2926 cycles) and five donor oocyte studies (7648 cycles). LBR rate was found to be significantly increased when male age was under 40 years in autologous oocyte studies (OR 2.37, [1.15 – 4.85]; p = 0.02) but no difference in LBR was found in donor oocyte studies (OR1.03, [0.80 – 1.32]; p = 0.84). Miscarriage rate was reported in two autologous oocyte studies (970 cycles) and four donor oocyte studies (3741 cycles). Miscarriage was found to be more likely with male age over 40 years in autologous studies (OR 0.57, [0.42 – 0.77]; p = 0.0003). In donor oocyte studies there was a trend towards increased miscarriage when male age was above 50 years however this effect did not reach statistical significance (OR 0.71, [0.48 – 1.04]; p value = 0.08).
Limitations, reasons for caution: The autologous oocyte studies are relatively heterogeneous in populations with varied female ovarian reserve and differing male age group divisions. The analysis was limited to cycles including women under 39 years of age to mitigate this heterogeneity.
Wider implications of the findings: Men over the age of 40 may have a reduced chance of a successful outcome following ART when using autologous oocytes. This effect does not appear to be present when donor oocytes are studied although men over the age of 50 years may still have an increased chance of miscarriage.
Trial registration number: n/a

P-045 Microfluidics is highly effective in selecting a sperm subpopulation with low DNA fragmentation index.

K. Wouters[1], L. Vandenberghe[1], A. Racca[1], D. Jankovic[1], G. Verheyen[1], I. Mateizel[1]

[1]UZ Brussel, Centre for reproductive medicine, Brussel, Belgium

Study question: Is microfluidic sorting of unprocessed semen better in selecting non-DNA fragmented sperm compared with other semen preparation techniques?
Summary answer: The use of microfluidic sperm sorting chip allows the selection of highly motile sperm with very low levels of DNA fragmentation.
What is known already: DNA fragmentation represents the last event of cell apoptosis. The use of sperm cells with DNA fragmentation for oocyte insemination is correlated with a negative paternal effect on embryo development and an increased miscarriage rates.

Both microfluidic sorting (MS) of unprocessed semen and Magnetic activated cell sorting (MACS) performed after density gradient centrifugation were reported to improve the sperm quality by selecting non apoptotic cells. However, it is uncertain which procedure leads to the lowest DNA fragmentation index (DFI).
Study design, size, duration: The study investigates whether microfluidic sorting of unprocessed semen improves sperm selection as compared to standard preparation procedures (sperm washing and density gradient centrifugation) or MACS in split samples after routine diagnostic semen analysis. The primary outcome was sperm DNA fragmentation index and the secondary outcome was sperm motility.
Participants/materials, setting, methods: Sperm samples from 6 different patients were used. Each sample was split and subjected to 4 different sperm selection techniques: 1) sperm washing (SW), 2) density gradient centrifugation (DGC), 3) DGC followed by MACS (Miltenyi Biotec, Germany) and 4) MS (Fertile plus, Koek Biotechnology).
DNA fragmentation was analyzed using the Sperm-Chromatin-Dispersion Assay (GoldCyto sperm kit, Goldcyto Biotech corp.). The DFI was calculated on at least 500 cells by the Sperm Class Analyzer CASA-system (Microptic, Spain).
Main results and the role of chance: The median DFI for the 4 processing techniques were: 18.3% (range 13.9-87.6) for SW; 14.6% (range 6.1-71.7) for DGC; 11.8% (range 3.9-46.9) for MACS, and MS: 0.7% (range 0-34.2) for MS. The samples processed by microfluidics showed a significantly lower DNA fragmentation rate compared to the other techniques (Friedman test, P<0.05).
The median progressive motility for the 4 processing techniques were: 47.5% (range 29-54) for SW; 58% (range 27-61) for DGC; 46% (range 3-78) for MACS and 92.5% (range 82-96) for MS.

The samples processed by microfluidics showed a significantly higher progressive motility (Friedman test, P<0.05).
Limitations, reasons for caution: The major limitation of the study is the low sample size although the advantage of MS is obvious. Consequently, there is a lack of variation between the samples regarding sperm quality. As this observational study was conducted on semen samples for diagnostic analysis, data on clinical outcome are not available.
Wider implications of the findings: Microfluidic sorting of sperm selects a population with significantly lower DNA fragmentation index and higher proportion of progressive motility compared to standard selection methods or MACS. Moreover, MS offers the advantage of using unprocessed semen, as such reducing the negative impact of centrifugation as compared to standard sperm selection methods.
Trial registration number: not apllicable

P-046 Enzymatic digestion by Collagenase-IV improves quality of testicular sperm retrieved in Non-Obstructive Azoospermic (NOA) patients

E. Piatti[1,2], E.M. Colpi[3], G. Caminiti[2], F. Castiglioni[2,4], E. Bernasconi[5], P. Testa[5], V. Monti[5], M. Bellavia[4], G. Momi[6], G.M. Colpi[2,4]

[1]ProCrea, Andrology, Lugano, Switzerland ; [2]Clinica San Carlo, Andrology, Paderno Dugnano, Italy ; [3]Gynecentro Ticino, Gynaecology, Lugano, Switzerland ; [4]ProCrea, Reproductive Unit, Lugano, Switzerland ; [5]Clinica San Carlo, Reproductive Unit, Paderno Dugnano, Italy ; [6]ProCrea, IVF Laboratory, Lugano, Switzerland

Study question: Does Enzymatic Digestion alone have positive effects on isolation of human sperm from surgical testicular samples, compared to standard mechanical treatment?
Summary answer: Enzymatic Digestion alone by Collagenase-IV seems a valid method to increase the total surgically retrieved viable sperm count suitable for ICSI.

What is known already: When available, surgically-retrieved testicular-sperm (Testi-Sperm) used with ICSI offer an opportunity to father children even to NOA patients. The treatment of testicular fragments is crucial, both for a positive retrieval and for the total Testi-Sperm number obtained and storable for ICSI. Enzymatic treatment following mechanical mincing of testis fragments was reported to increase retrieval success rate and final Testi-Sperm count. Collagenase-IV, a Sertolian protease, was shown to provide higher yelds of Testi-Sperm cells. However, a critical factor for ICSI success is sperm motility, as a proof of sperm viability, especially after cryopreservation.
Study design, size, duration: A prospective blind observational study was performed on 24 NOA patients. In each, the testicular microfragments from microdissection-TESE were divided into two same-weight parts: the first underwent mechanical mincing, the second was treated by Collagenase-IV solution alone (never previously reported). Viability was assessed by Eosin-Y test (WHO 2010), applied to small cell suspension aliquots; and concentration by a counting chamber. All samples were prepared by one biologist and then blind-evaluated by another.
Participants/materials, setting, methods: The surgical samples were placed in Petri dishes containing HAM's F10 medium: the first half, mechanically minced to obtain a homogeneous cell suspension, and the second half, immersed in 1 ml Collagenase IV solution. Both were incubated at 37°C for 120'; then, both treatments were stopped by adding 5 ml HSA 0.5% medium, the tubes centrifuged for 10' at 500g for washing, the pellets re-suspended with fresh medium and analyzed.
Main results and the role of chance: For each treatment, we measured the Total Count of the Testi-Sperm retrieved, the percentage of Viable Testi-Sperm (by Eosin-Y test on over 100 cells), and consequently the Total Count of Viable Testi-Sperm actually available for cryopreservation or ICSI. Statistical analysis was performed by Wilcoxon-paired rank test.

Viable Testi-Sperm were [X±SD; (range)]: 31.9±9.7% (19-63%) and 67.2±8.1% (52-80%) for mechanically and Collagenase-IV treated samples, respectively ($p<0.01$).

Total Testi-Sperm retrieved were [M (range)] $0.49 \cdot 10^6$ ($0.06\text{-}2.78 \cdot 10^6$) and $0.77 \cdot 10^6$ ($0.14\text{-}2.3 \cdot 10^6$)($p<0.01$), and Total Viable Testi-Sperm retrieved were $0.16 \cdot 10^6$ ($0.01\text{-}1.0 \cdot 10^6$) and $0.54 \cdot 10^6$($0.08\text{-}1.59 \cdot 10^6$) ($p<0.01$) for mechanically and Collagenase-IV treated samples, respectively.

Therefore, Collagenase-IV treatment alone gives a Total Count of Viable Testi-Sperm useful for cryopreservation or ICSI significantly higher than that of the standard mechanical treatment.
Limitations, reasons for caution: Our results on the higher Viable Total Testi-Sperm Count retrieved by Collagenase-IV alone versus mechanical treatment invite future studies on microdissection-TESE samples, especially to confirm the maintained functional competence of Testi-Sperm in ICSI.
Wider implications of the findings: The enzymatic-only method in processing Testi-Sperm during microdissection-TESE can offer higher positive Testi-Sperm retrieval rates and higher number of viable cells available for cryopreservation or ICSI in NOA patients, especially in those with the poorer prognosis.
Trial registration number: None

P-047 Multiparametric comparative analysis of the effectiveness of conventional ICSI and physiological ICSI (PICSI) among couples with male infertility factor

D. Rusakov[1], O. Shurygina[2], A. Petrova[3], T. Bykova[3], A. Baizarova[3]

[1]Samara state medical University- IDK medical company, Department of histology and embryology- ART Department, Samara, Russia C.I.S. ;
[2]Samara state medical University- IDK medical company, Department of histology and embryology - Department of reproductive medicine clinical embryology and genetics - ART Department, Samara, Russia C.I.S. ;
[3]IDK medical company, ART Department, Samara, Russia C.I.S.

Study question: Multiparametric comparative analysis of the effectiveness of conventional ICSI and physiological ICSI (PICSI) in couples with male infertility factor performed.
Summary answer: PICSI compared to ICSI reduces the number of embryo losses at the laboratory stage and increases cumulative pregnancy rate and cumulative live-birth rate.
What is known already: According to the Russian Association of human reproduction data after the transfer of embryos obtained by ICSI pregnancy occurs in 36.2 % of cases and perinatal miscarriage reaches 78.1 %. The use of hyaluronate for the study of male germ cells in the ICSI procedure allows selecting of sperm without DNA damage and abnormalities in the chromosomal set, thereby increasing the chances of successful fertilization and meanwhile according to a large systematic review devoted to comparing the effectiveness of ICSI and PICSI in male infertility factor, which included 2918 studies, there are no statistically significant differences between these two methods.
Study design, size, duration: Retrospective multiparametric comparative analysis of 284 ICSI procedures and 200 PICSI procedures performed from 2015 to 2019 in the ART Department of "IDK Medical company", (Samara, Russia) was performed.
Participants/materials, setting, methods: The following performance parameters were evaluated: fertilization rate, blastocyst formation rate, embryo freezing rate, embryo utilization rate, implantation frequency rate, pregnancy rate, live-birth rate, cumulative pregnancy rate, cumulative live-birth rate.
Main results and the role of chance: The fertilization rate in ICSI was 86%, in PICSI - 82%. The embryo freezing rate and blastocyst formation rate in ICSI were 28% and 47% respectively, in PICSI - 33% and 51%. The differences between two methods are statistically insignificant ($p>0,05$). The embryo utilization rate in ICSI was 48%, in PICSI - 58%. The differences in these indicators are statistically significant ($p=0,043$). While assessing the implantation frequency rate it was found that in ICSI it has become 44.6%, in PICSI it has become 45%. The pregnancy rate and the live-birth rate after ICSI were 47% and 33% respectively, and the pregnancy rate and the live-birth after PICSI were 48.5% and 35% respectively. The differences in these indicators are statistically insignificant ($p>0,05$). While analyzing the cumulative pregnancy rate and the cumulative live-birth rate the following data was provided: in ICSI these indicators were 53% and 34%, respectively, in PICSI - 81.5% and 56.5% respectively. The differences in these indicators are statistically significant ($p=0,038$).
Limitations, reasons for caution: Absent.
Wider implications of the findings: Multiparametric analysis makes it possible to evaluate the effectiveness of ART programs with great confidence. There are no significant differences between ICSI and PICSI for most of the analyzed indicators, which is consistent with the literature data. But cumulative performance indicators demonstrate the advantage of PICSI over conventional ICSI.
Trial registration number: not applicable

P-048 Which is better for sperm incubation before IVF: lower or higher oxygen?

E. Yalcinkaya[1], B. Alyuruk[2], F. Martinez Pastor[3]

[1]Adatip Hospital, Assisted Reproduction Unit, İstanbul, Turkey ;
[2]Kocaeli University, IVF Unit, Kocaeli, Turkey ;
[3]INDEGSAL, University of Leon, Leon, Spain

Study question: Do lower levels of oxygen result in less oxidative stress on sperm cells before fertilization in bull?
Summary answer: This pilot study reported a higher oxidative stress due to increased mitochondrial activity under high oxygen during incubation.
What is known already: Fertilization of bovine oocytes is still carried out under high oxygen levels in most of the laboratories although embryo culture is performed under lower oxygen tension due to the solid evidence supporting higher blastocyst development rates. However, incubation of sperm cells under lower oxygen concentration might be crucial in terms of sperm function considering that these cells are susceptible to oxidative stress due to their inadequate cell repair system against excessive production of reactive oxygen species (ROS).
Study design, size, duration: This was a pilot study for comparing the effects of two different levels of O_2 on sperm cells during capacitation. The sample of the study included 10 cryopreserved bull semen samples from different breeders. Treatment groups were named as LO (low oxygen; 5% O_2) and HO (high oxygen; 20% O_2).
Participants/materials, setting, methods: Two straws were thawed per each bull and sperm cells were left to swim up for 1 hour. Then, swam sperm cells were divided equally into two; and aliquots were assigned to both treatment goups to incubate for 5-6 hours. Variables of motility, progressive motility, mitochondrial activity, capacitation status and intracellular ROS were measured by flow cytometry at 0 h and 5-6 h.

Main results and the role of chance: Compared to starting levels, both groups showed significantly decreased total and progessive motility, nonsignificant increase in capacitation status, significant decrease in the percentage of active mitochondria and statistically significant decreases in intracellular ROS. The decrease in total and progressive motility was found to be significantly higher in LO compared to HO (22.6% vs 32.8% and 24.4% vs 18.6%; $p<0.05$, respectively). Moreover, the percentage of capacitated cells were found to be higher in HO compared to LO; but the difference was not statistically significant ($p>0.05$). At the end of incubation, mitochondrial activity was observed to be slightly higher in HO than LO; but the difference was not again statistically significant ($p>0.05$). Interestingly, intracellular ROS was found to be decreased following incubation; and it was found to be statistically lower in LO group compared to HO ($p<0.05$).
Limitations, reasons for caution: The main limitation of our study was bull variability and the limited size of the study sample.
Wider implications of the findings: Considering that ROS may lead to problems in reproductive processes when present in excess amounts, use of lower oxygen also during capacitation was found to keep intracellular ROS levels significantly lower as desired. Any further effects on the quality of spermatozoa need to be evaluated by fertilization and IVF outcomes.
Trial registration number: not applicable

P-049 In vitro myo-inositol treatment significantly ameliorates motility, mitochondrial respiratory efficiency and DNA integrity of human sperm

P. Piomboni[1], R. Ponchia[1], B. Semplici[1], L. Governini[1], P. Artini[2], E. Casarosa[2], I. Marzi[2], A. Luddi[1]

[1]Siena University, Molecular and Developmental Medicine, Siena, Italy ; [2]University of Pisa, Experimental and Clinical Medicine, Pisa, Italy

Study question: Does the *in vitro* treatment with myo-inositol affects the mitochondrial function and DNA integrity of human sperm in both basal and capacitated condition?
Summary answer: The *in vitro* treatment with myo-inositol of normozoospermic samples, before and after swim-up capacitation, leads to a significant amelioration of sperm motility and DNA integrity
What is known already: Mammalian fertilization is a multifaceted and complex process because the site of semen deposition is far from the site of fertilization and sperm need to have an adequate motility to reach the oocyte. Sperm motility, as well as capacitation, hyperactivation and acrosome reaction, requires energy and, to this regard, mitochondria play a pivotal role in the aerobic production of energy in comparison with the glycolytic pathway. At same time, DNA integrity must be preserved during sperm capacitation and related processes. These features may be affected by in vitro procedure for sperm selection and capacitation in assisted reproductive techniques.
Study design, size, duration: Human ejaculated sperm were analyzed according to WHO guidelines between September 2019 - January 2020; a total of 40 normozoospermic samples were selected in order to evaluate the effect of an *in vitro* treatment with myo-inositol, before and after swim up selection. Isolated sperm from all specimens were used to measure the respiratory control ratio (RCR) and the DNA oxidation; Sperm ultrastructure was analysed. All patients had given their informed consent.
Participants/materials, setting, methods: Normozoospermic human semen samples (n=40) collected at the Siena University Hospital were processed for the oxygraphic analysis, before and after swim up selection, with or without treatment myo-inositol (2 or 20 µg/µl). To this end, sperm cells were demembranated by hypotonic swelling and the RCR was calculated. DNA sperm oxidation measurement by 8-OHdG and ultrastructural evaluation by transmission electron microscopy were performed in myo-inositol treated and untreated sperm samples.
Main results and the role of chance: Our data highlight that myo-inositol treatment significantly increased sperm progressive motility, in a dose dependent manner ($p<0.05$), both in basal and swim-up selected samples. Moreover, the consumption of O_2 measured by oxigraphic analysis was significantly raised by increasing doses of myo-inositol *in vitro* treatment.The concentration of 8-OHdG, an early marker of DNA oxidation, in untreated sperm was significantly higher than those of sperm supplemented with myo-inositol, confirming its protective role against DNA oxidative damage during the *in vitro* manipulation/incubation of human sperm. To evaluate any possible ultrastructural modifications, semen samples treated with myo-inositol or untreated were analysed by transmission electron microscopy. Despite the treatment seems to not induce appreciable ultrastructural changes, it may be highlighted that sperm surface as well as the observed environment are clearly free from any amorphous fibrous material, which instead appears as background in untreated sperm samples. This finding may confirm the ability of myo-inositol to disrupt the mucoid masses of the seminal fluid, usually able to entrap sperm and any other seminal fluid components, thus reducing sperm motility and capacitation efficiency.
Limitations, reasons for caution: Large-scale studies should be required to assess whether an addition of myo-inositol improves sperm motility, also in oligoasthenozoospermic men. Moreover, the mechanism by which myo-inositol ameliorates mitochondrial activity should be further evaluated.
Wider implications of the findings: These data, if confirmed in future larger studies, will disclose that myo-inositol ameliorates key feature of human sperm (motility, mitochondrial functions, DNA integrity).
Trial registration number: None

P-050 Impact of sperm quality to achieve pregnancies in oocyte donation cycles

M. Cozzolino[1], I. Hervás[1], R. Rivera-Egea[1], A. Pellicer[1], N. Garrido[1]

[1]Fundacion IVI, Valencia, Valencia, Spain

Study question: When should be considered sperm replacement in case of the first failure of oocyte donation?
Summary answer: Replacement patients by donors sperm significantly increase live birth rate and cumulative live birth rate (CLBR) either in oligo-asthenoteratozoospermia or in suboptimal/normal sperm.
What is known already: Oocyte donation is an extremely successful treatment in assisted reproductive technology (ART), unfortunately, any couples remain still barren despite normal sperm and regular uterus morphology. The use of ICSI in clinical practice has modified the indications of sperm donation use, currently, the ESHRE suggests the donation of sperm after at least three failed cycles of ICSI with the sperm of the couple. A repetitive clinical question that remains unsolved so far, concerns to when a couple should be recommended to change patient's by donor sperm after oocyte donation failed treatments
Study design, size, duration: A retrospective study in university-affiliated ART centers was conducted between January 2000 and April 2019. 6065 cycles of oocyte donation were included in the study, with a total of 7757 embryo transfers and 14119 embryos transferred.
Participants/materials, setting, methods: Couples in which male partners have oligoasthenoteratospermia or suboptimal/normal sperm were scheduled for oocyte donation cycles after the failed first attempt, using patients or donors sperm.Oligoasthenoteratospermia was defined as the presence of less than 5 million total progressive motile spermatozoa in the ejaculate. Obstructive azoospermia was excluded. Live birth rates per embryos transferred and embryo transfers, and cumulative live birth rates (CLBR) considering oocyte consumed in the previous donation cycles using patient or donor sperm.
Main results and the role of chance: After the first failure of the oocyte donation cycle our results confirm that in patients with oligoasthenoteratospermia or suboptimal/normal sperm, replacement patient's sperm by donor sperm as soon as increased live birth per embryo transfers or the number of embryos transferred. The live birth rate in couples with oligoasthenoteratospermia showed that replacement patient by donor sperm OR 2.18 CI95% (1.7-2.8) $p<0.001$ when considered for embryo transfers and OR 2.46, CI95% (1.9-3.2) $p<0.001$ considering for embryos transferred. In couples with suboptimal/normal sperm replacement of patient sperm with donor showed an increase live birth for embryo transfers OR 2.21, 95%CI (1.7-2.8), $p<0.001$ and for embryos transferred OR 2.43, CI 95%(1.9-3.1), $p<0.001$. The same results were reached in the third cycles of donation with improving live birth rate for embryo transfers OR 1.51, CI95% (1.0-2.2), $p=0.033$ and embryos transferred OR 1.77, CI 95% (1.2-2.6), $p=0.004$ in oligoasthenoteratospermia, in case of suboptimal/normal sperm was OR 1.40, CI95%(1.0-2.0), $p=0.039$ for embryo transfer and OR 1.53, CI 95%(1.0-2.4), $p=0.013$ for embryo transferred.The survival curves of CLBR with the patient and donor sperm considering oocyte by oocyte showed a statistically significant difference ($p<0.001$), replacing sperm patients after fifteen in oligoasthenoteratospermia and twenty oocytes donated, in suboptimal/normal sperm.

Limitations, reasons for caution: The principal limitation of our study was the lack of andrological studies at the molecular level, such as the sperm DNA fragmentation test or sperm FISH test, which could have identified a clearer indication for the gamete change for each patient and the retrospective nature of the study.
Wider implications of the findings: Prospective studies taking into account an andrological study should confirm the possibility of changing the patient's semen after the first failed donation.
Trial registration number: not applicable

P-051 Distinct expression levels of spermatogenic markers and growth factors in testicular tissue of Klinefelter patients with negative sperm retrieval

E. Lunenfeld[1], M. Azab[2], S. Kleiman[3], R.R. Hauser[4], M. Huleihel.[2]

[1]The Center of Advanced Research and Education in Reproduction CARER-Faculty of Health Sciences Ben-Gurion University of the Negev- Beer-Sheva- Israel. and Soroka Medical Center, Department of Obstetrics and Gynecology, Beer - Sheva, Israel ;
[2]The Center of Advanced Research and Education in Reproduction CARER- Faculty of Health Sciences- Ben Gurion University of the Negev, The Shraga Segal Departement. of Microbiology- Immunology and Genetics-, Beer Sheva, Israel ;
[3]Male Fertility Clinic and Sperm Bank- Lis Maternity Hospital- Tel Aviv Sourasky Medical Center School of Medicine Tel Aviv University, Obstetrics and Gynecology, Tel Aviv, Israel ;
[4]Male Fertility Clinic and Sperm Bank- Lis Maternity Hospital- Tel Aviv Sourasky Medical Center- School of Medicine Tel Aviv University, Obstetrics and Gynecology, Tel Aviv, Israel

Study question: Can we use spermatogenic markers and testicular growth factors to predict spermatogenesis in testicular biopsies of Klinefelter patients without sperm?
Summary answer: The expression of oct4 and CSF-1 were high and in a positive correlation with the expression of crem-1 and protamine, in the examined biopsies.
What is known already: Klinefelter syndrome (KS) is the most prevalent genetic disorder, occurring at about 0.2% of males and 11% of the azoospermic patients. At birth, KS patients have normal amount of testicular germ cells. Until age 10, spermatogonial cells (SCs) are present in the testes, but can be found in 40-50% of the adult patients. The presence of SCs (OCT4 and MAGE-A4) was reported. It is suggested that spermatogenic arrest occurs at the spermatogonium or primary spermatocyte level in KS boys, which undergo apoptosis instead of differentiation. The expression of growth factors in testicular biopsies of Klinefelter patients is not yet clear.
Study design, size, duration: The expression levels of the pre-meiotic (oct4, cd9, gfr-a1, a-6-integrin, sall4, c-kit), meiotic (crem-1) and post-meiotic (protamine) markers as well as the colony stimulating factor-1 (CSF-1) (growth factor) were examined in testicular biopsies of 17 Klinefelter patients (28-40 years old), with no sperm in their testicular tissue.
Participants/materials, setting, methods: 17 Klinefelter patients underwent conventional TESE and no sperm was retrieved. qPCR analysis was used to quantify the expression levels of the extracted RNA from the 17 examined biopsies in whom sperm was not retrieved. The levels of the markers were expressed as fold of increase compared to GAPDH in the same tissue sample.
Main results and the role of chance: Our results show that in 14 biopsies, we examined low expression levels of oct4 - a pre-miotic marker (fold of increase was less than 5), CREM - a miotic marker (fold of increase was less than 6) and CSF-1 (fold of increase were less than 18) but no expression of protamine. However, in three biopsies that highly expressed oct4 (29, 39 and 61 fold) and CSF-1 (162, 86 and 88 fold, respectively), the expression levels of crem-1 were high (50, 21 and 28 fold, respectively) and also protamine (1364, 20 and 466, respectively). No such correlation was found between the expression levels of the pre-meiotic markers cd9, gfr-a1, a-6-integrin, sall4, c-kit and the expression levels of the meiotic marker crem-1 and the post-meiotic marker protamine.
Limitations, reasons for caution: The number of the patients need to be increased and the expression levels of the markers need to be confirmed by immunostaining.
Wider implications of the findings: We would recommend to verify pathological results using those markers in order to increase precision diagnosis among NOA patient in whom sperm was not retrieved. These results may be used for considering re-TESE or re-microTESE.
Trial registration number: not required

P-052 Outcome of IVF/ICSI procedures in male cancer patients: retrospective analysis of procedures performed between 2004 – 2018

T. Papler[1], M. Štimpfel[2], E. Vrtacnik Bokal[3], S. Drobnič[2]

[1]University Medical Center Ljubljana, Department of Human Reproduction, Ljubljana, Slovenia ;
[2]University Medical Centre Ljubljana, Department of Human Reproduction, Ljubljana, Slovenia ;
[3]Univeristy Medical Centre Ljubljana, Department of Human Reproduction, Ljubljana, Slovenia

Study question: Study question: Is the clinical outcome of IVF/ICSI cycles comparable when fresh or cryopreserved semen from male cancer patients is used for fertilization?
Summary answer: There are no statistically significant differences in clinical outcomes of IVF/ICSI procedures when fresh or frozen semen is being used
What is known already: Due to improved survival of men after oncological treatment it has become important to enable them a quality life after the treatment is finished. Fertility preservation and a chance to have biological children represent an important aspect of quality of life. In men we can preserve fertility by cryopreserving semen prior to the start of oncological treatment. Outcomes of IVF procedures are comparable when we use fresh or frozen semen in healthy infertile couples. Data on the use of fresh or frozen semen from oncological patients and the outcomes of IVF procedures however, are scarce.
Study design, size, duration: We retrospectively analysed the outcome of IVF/ICSI cycles where cryopreserved or fresh semen from male oncological patients was used for oocyte fertilization. Analysis included 226 IVF/ICSI cycles performed between 2004 and 2018, 138 with cryopreserved semen and 88 with fresh semen, respectively. We had no exclusion criteria with regard to female age (aged 23-43 years) or female causes of infertility.
Participants/materials, setting, methods: Groups (cryopreserved versus fresh semen) were compared in terms of fertilization rate, number of embryos, blastocysts and cryopreserved embryos. Pregnancy, live birth and miscarriage rates were determined to determine which type of semen used yields better clinical outcomes. To determine the differences between the two groups, Pearson's chi-square test and two-tailed t-test were used. Statistical significance was set at $p < 0.05$.
Main results and the role of chance: The cryopreserved semen and fresh semen group of patients were comparable for mean female age (31.7 ± 4.7 vs. 32.5 ± 4.3; p=0.65), mean number of retrieved oocytes per aspiration (10.2 ± 5.6 vs. 10.1 ± 6.6; p=0.71), fertilization rate (56.9% vs. 59.1%; p=0.35) and number of embryos (629 vs. 423; p=0.34). There was however, statistically significant higher number of blastocysts (211 vs 116; **p=0.018**) and cryopreserved embryos (124 vs 51; **p=0.001**) in the group where cryopreserved semen was used. In terms of clinical IVF outcomes, there were no differences in pregnancy (34.2% vs. 28.6%; p=0.43), live birth (24.8% vs. 24.3%; p=0.92) and miscarriage (27.5% vs. 10%; p=0.18) rates between cryopreserved and fresh semen group.
Limitations, reasons for caution: In the fresh semen group, timing from finished oncological treatment to IVF procedure should be taken into account to get a clearer perspective about semen quality. Also, the type of cancer and oncological treatment should be considered to get a clearer picture.
Wider implications of the findings: Semen cryopreservation prior to oncological treatment represents an efficient way for fertility preservation in men with cancer. Furthermore, it seems that the use of cryopreserved semen is better in terms of number of blastocysts obtained and number of embryos cryopreserved after IVF procedure.
Trial registration number: Retrospective analysis.

P-053 Impact of slow freezing on sperm nuclear quality and telomere length

C. Gouhier[1], H. Pons-Rejraji[1,2], S. Carlet-Dollet[2], B. Pereira[3], A. Tchirkov[4], F. Brugnon[1,2]

[1]CHU Clermont-Ferrand- Hôpital Estaing-, Laboratoire de Biologie du Développement et de la Reproduction- AMP- CECOS, Clermont-Ferrand, France ; [2]Faculté de Médecine, Université Clermont Auvergne- Inserm 1240- IMoST, Clermont-Ferrand, France ; [3]CHU Clermont-Ferrand, DRCI- Délégation Recherche Clinique et Innovation, Clermont-Ferrand, France ; [4]CHU Clermont-Ferrand- Hôpital Estaing-, Service de Cytogénétique Médicale, Clermont-Ferrand, France

Study question: Does slow freezing affect human sperm telomere length?
Summary answer: Slow freezing of human sperm does not alter telomere length despite of increasing DNA oxidation and fragmentation.
What is known already: Sperm telomere length (STL) is a potential interesting biological marker to assess male fertility. While its relationship with standard sperm parameters is still controversial, recent studies have shown correlations between STL and DNA alterations, notably DNA fragmentation and oxidation. Likewise, STL is positively related with pregnancy rate. As STL is transmitted to offspring, its alteration could lead to pathologies that may worsen from generation to generation. Human sperm freezing is an indispensable tool for male fertility preservation despite of inducing nuclear spermatic alterations. Nevertheless, nothing is known about its impact on STL.
Study design, size, duration: This prospective study was conducted between January and December 2018 and approved by the "Germethèque" Scientific Committee and the Ethical Committee under the French IRB CPP Sud-Est6. Samples were surplus semen obtained from 63 men undergoing routine semen analysis at the Center for Reproductive Medicine. STL measurement was carried out on all the 63 fresh samples, and for 30 of those also after cryopreservation. In addition, nuclear quality was measured before and after freezing-thawing cycle.
Participants/materials, setting, methods: The 63 subjects (35 normozoospermic) had a mean age of 34±0.6 years and a BMI of 27.4±0.8. Spermatozoa were slowly frozen in high security straws using freezing medium and programmable freezer. STL was measured for all fresh and frozen-thawed samples by qPCR. For 10 samples, STL before and after freezing-thawing was additionally assessed by qFISH. For another 10, sperm DNA oxidation (8-OHdG residues immuno-detection) and fragmentation (TUNEL assay) were measured by flow cytometry.
Main results and the role of chance: We did not measure any significant impact of freezing on the mean telomere length of sperm samples, whether it was analyzed by qPCR (3.25 ± 0.15 arbitrary unit (a.u.) *vs.* 3.46 ± 0.2 a.u., n = 30) or by qFISH (0.84 ± 0.1 a.u *vs.* 0.84 ± 0.04 a.u, n=10). qFISH method highlighted STL heterogeneity within each sperm sample. The cryopreservation tended to modify STL distribution (segmented by intervals of 0.25 a.u.) even if it was not statistically significant.

In addition, slow freezing-thawing cycle decreased sperm motility and vitality ($p < 0.05$ for both, n=10), and increased DNA oxidation (average oxidation intensity 839 ± 210 a.u. *vs.* 1445.2 ± 202 a.u., $p < 0.05$, n=10) and fragmentation (23.1 ± 5.4 % *vs.* 39 ± 6 %, $p < 0.005$, n=10). Finally, we did not measure any significant correlations between STL and the analyzed spermatic nuclear markers.
Limitations, reasons for caution: Standard parameters of analyzed samples were normal or slightly altered because the different measurements required a high number of spermatozoa. Nevertheless, fertility preservation is proposed to men with potentially altered sperm parameters, therefore it would be essential to measure cryopreservation impact on semen of oligo-astheno-teratozoospermic patients.
Wider implications of the findings: Although slow freezing increased DNA oxidation and fragmentation, it did not alter STL. To our knowledge, this was the first study to assess the impact of cryopreservation on STL. Compared to qPCR that only gives a mean STL, qFISH provides the distribution of STL within a semen sample.
Trial registration number: not applicable

P-054 Relationship between the degree of sperm-autoimmunization with natural and intra-uterine insemination-assisted live births

S. D'Andrea[1], C. Castellini[1], E. Minaldi[1], M. Totaro[1], G. Cordeschi[1], S. Francavilla[1], F. Francavilla[1], A. Barbonetti[1]

[1]University of L'Aquila, Pubblic Health- Clinical- Life and Envirenomnental Science, L'Aquila, Italy

Study question: What is the relationship between the degree of sperm-autoimmunization, assessed by IgG-mixed antiglobulin reaction (MAR) test, with natural and intra-uterine insemination (IUI)-assisted live births?
Summary answer: Infertile couples with 100%-positive MAR-test, where natural live births were much less than at lower degree of positivity (50%-99%), could be successfully treated with IUI.
What is known already: Although the World Health Organization (WHO) has recommended the IgG-MAR test as an integral part of semen analysis, considering 50% antibody-coated motile spermatozoa as the clinically-relevant threshold, the predictive value of the degree of positivity of the MAR test above such a cut-off on the occurrence of natural pregnancies still remains largely undetermined. Furthermore, the effectiveness of IUI in cases of strong sperm-autoimmunization is not yet well-established.
Study design, size, duration: This was a historical cohort study on 108 infertile couples with ≥50% positive MAR test in men, who had attended a university/hospital's andrology/infertility clinic for the management of couple infertility from March 1994 to September 2017.
Participants/materials, setting, methods: The IgG-MAR was carried out as an integral part of semen analysis. The patients were divided into two groups: 100%– and 50%–99%-positive MAR test. Post-coital test (PCT) was performed in all the couples and IUI was offered as the first-line treatment. Laboratory and other clinical data were retrieved from the computerized database. Data on subsequent pregnancies were obtained by contacting patients over the telephone.
Main results and the role of chance: Eighty-four men (77.8%) were successfully contacted by telephone and agreed to participate. Forty-four men belonged to the group of 100%-positive MAR test, while 40 showed lower MAR test positivity. The couples with a 100%-positive MAR test showed a natural live birth rate per couple (LBRcouple), that was considerably lower than that observed at a lower degree of positivity (4.5% vs. 30.0%; $p = 0.00001$). Among the clinical variables, a significant difference was observed only for the PCT outcome, which was poorer in the 100%-positive MAR test group. Better PCT outcomes (categorized as negative, subnormal and good) were positively correlated to the occurrence of natural live births (6.3%, 21.7%, and 46.2%, respectively; $p = 0.0005$ for trend), for which the sole independent negative predictor was the degree of sperm-autoimmunization. IUI was performed as the first line treatment in 38 out of 44 couples with 100%-positive MAR test, yielding 14 live births (LBRcouple = 36.8%). In couples with lower MAR test positivity, the LBRcouple after IUI was similar to the natural LBRcouple (26.9%).
Limitations, reasons for caution: We cannot exclude uncontrolled variables that may have affected natural pregnancies during the follow-up, or a selection bias in the comparison of outcomes, due to the lack of randomization even though the clinical variables were not different in couples that had or had not undergone IUI.
Wider implications of the findings: While 100%-positive IgG-MAR test can represent the sole cause of a couple's infertility, which could be successfully treated with IUI, a lower degree of positivity may only represent a contributing factor to be considered in addition to conventional prognostic factors, including the PCT outcome, in decision making "treat or wait".
Trial registration number: not applicable

P-055 Cell-free seminal mRNA as a marker of complete obstruction in azoospermic men

M. Jeseta[1], P. Ventruba[1], W. Kranc[2], K. Jopek[3], J. Žáková[1], I. Crha[1,4], B. Kempisty[2,3]

[1]Faculty of Medicine- Masaryk University Brno and University Hospital Brno, Department of Gynecology and Obstetrics, Brno, Czech Republic ; [2]Poznan University of Medical Sciences, Department of Anatomy, Poznan, Poland ; [3]Poznan University of Medical Sciences, Department of Histology and Embryology, Poznan, Poland ; [4]Faculty of Medicine- Masaryk University Brno and University Hospital Brno, Department of Nursing and Midwifery, Brno, Czech Republic

Study question: Is it possible to use cell-free mRNA (cfs-mRNA) in seminal plasma as a diagnostic biomarker of obstruction in azoospermic patients?
Summary answer: Our results showed that the seminal plasma samples from non-obstructive azoospermic donors differed significantly in contents of cell-free mRNA when compared to obstructive azoospermic men.
What is known already: Extracellular cell-free mRNA has been detected in various body fluids including seminal plasma. This mRNA is actively released from living cells or can be released from dying cells. Cell-free seminal mRNA (cfs-mRNA) can be used as a biomarker in many non-invasive diagnostic applications as it contains tissue-specific and stable mRNA. In azoospermic males, it is very important to know if there is an obstructive problem or not. However, to the best of our knowledge there is no existing model that can accurately predict successful sperm retrieval by MESA or TESE. Analysis of cfs-mRNA is a promising tool for such prediction.
Study design, size, duration: In this study we evaluated cfs-mRNA composition in samples of seminal plasmas from men with non-obstructive azoospermia (NOA) and obstructive azoospermia (OA). A total of 8 patients (4-NOA and 4-OA) aged 28 to 40 years were included in this prospective study from spring to autumn 2019. The seminal plasma samples were collected after centrifugation of ejaculate. The cfs-mRNA content was determined by the microarray analyses.
Participants/materials, setting, methods: The non-obstructive azoospermia group included men who were cryptozoospermic in previous years and azoospermic in this analysis, the obstructive azoospermia group included men after vasectomy. RNA was isolated from a total of 8 samples. Two samples (with the highest concentration and quality) were selected for each group. Further analyses were performed using GeneChip ™ Human Gene 2.1 ST Array Strip microarrays. The statistical analysis was performed in the R programming language with the Bioconductor package.
Main results and the role of chance: Statistical analysis consisted of comparing cfs-mRNA content from men with non-obstructive azoospermia (NOA) and men with obstructive azoospermia (OA). In the next step, a total of 762 upregulated genes in NOA (fold above 2) were selected. Most of the upregulated genes belong to processes such as: cellular component organization or biogenesis (GO:0071840), cellular metabolic process (GO:0044237), cellular component organization (GO:0016043), organelle organization (GO:0006996). These processes belong to the GO BP (Gene Ontology Biological Process). The genes that showed the largest change in expression (largest fold) are these: *VTRNA1-1 (Vault RNA1-1), MT-TS1 (Mitochondrially Encoded tRNA Serine 1), MT-TV (Mitochondrially Encoded tRNA Valine), RNU6-441P (RNA, U6 Small Nuclear 441, Pseudogene), MT-TC (Transfer RNA Mitochondrial Cysteine), RNU1-83P (RNA, U1A Small Nuclear), PCGEM1(Prostate-specific gene), SEMG2 (Semenogelin 2), ND1 (NADH dehydrogenase, subunit 1), MT-TF (mitochondrially encoded tRNA phenylalanine)* and *PIP (prolactin induced protein)* which was previously considered as a specific protein marker of non-obstructive azoospermia.
Limitations, reasons for caution: A limitation is the number of samples included and analysed in this study which slightly reduced the power of statistical analysis. The obtained result will be validated on more samples using the quantitative RT-qPCR method.
Wider implications of the findings: These results suggest that the analysis of cell-free seminal mRNA is a very promising tool and that the cfs-mRNA could be used as a non-invasive biomarker for identifying the complete obstruction in case of azoospermia.
Trial registration number: Supported by MH CZ – DRO (FNBr, 65269705) and Ministry of Health, Czech Republic projects no. NV18-08-00291 and NV-18-08-00412.

P-056 Spermatogenesis in advanced-stage Hodgkin's lymphoma patients treated with escalated BEACOPP: a retrospective study of impaired fertility and recovery

E. Williams[1], V. Jayadev[1]

[1]*Concord Repatriation General Hospital, Andrology, Sydney, Australia*

Study question: To determine the effect of escalated-BEACOPP on spermatogenesis in Hodgkin's lymphoma patients and to investigate both the potential for spermatogenesis recovery and factors predicting recovery.
Summary answer: Escalated-BEACOPP causes significant spermatogenesis impairment in Hodgkin's lymphoma patients, with spermatogenesis recovery occurring only in a minority of individuals. No factors predicting recovery were elucidated.
What is known already: Escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone), a multiagent chemotherapy regimen, was introduced in the 1990s for the treatment of advanced-stage Hodgkin's lymphoma. It demonstrates superior prognostic outcomes in progression-free survival, overall survival and complete remission when compared to the preceding first-line protocol, ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine). These benefits over ABVD appear to come at the cost of increased germinal toxicity and impaired spermatogenesis in male patients. The degree of impairment and the recovery of this fertility post-treatment remains in question, and considering the youth of many Hodgkin's lymphoma survivors, is clinically significant.
Study design, size, duration: A retrospective longitudinal study examining semen and hormonal analysis of 28 male patients with advanced-stage Hodgkin's lymphoma (Ann Arbor stage IIB, III or IV) treated with escalated BEACOPP. Pre- (n=28) and post-treatment data (n=19) was evaluated. 19 patients engaged in post-escalated BEACOPP analysis which occurred at no uniform time, between one and six years post-treatment. The number of post-treatment analyses undertaken by each patient varied from one to four with a median of two.
Participants/materials, setting, methods: Included patients were those with histologically-confirmed advanced-stage Hodgkin's lymphoma who presented to Concord Repatriation General Hospital's (CRGH) Andrology Department between January 2009 and June 2017 for semen cryopreservation and analysis prior to commencing escalated-BEACOPP therapy, without age limits. As a retrospective study, data was obtained from medical records (stage, treatment) and semen cryopreservation data of the CRGH Andrology department. Each analysis involved seminal fluid analysis, hormonal analysis (testosterone, LH, FSH, SHBG) and testicular volume measurement.
Main results and the role of chance: In pre-treatment analysis, gonadal dysfunction was present in one in three patients with 68% of patients having normospermia and 32% having azoospermia or other dyspermia. In the identification of factors which may affect baseline sperm quality, only the presence of B symptoms (weight loss ≥ 10%, night sweats or fevers ≥ 38°C) was found to have significant effect on pre-treatment semen quality ($P<0.05$).

All 19 patients undergoing post-treatment follow-up were azoospermic at first analysis after escalated BEACOPP. Serum LH ($P<0.01$) and FSH ($P<0.001$) were significantly increased, while testosterone and SHBG concentrations were not significantly changed.

Three patients (15.8%) recovered spermatogenesis with a mean recovery time of 44 months. Spermatogenesis recovery in these three patients occurred at 36.5 months (Patient A), 44.6 months (Patient B) and 50.8 months (Patient C) post-treatment.

To ascertain whether potential predictors of spermatogenesis recovery exist, a binary logistic regression was performed on patient age (P=0.414), the presence of B symptoms (P=0.715), pre-treatment total sperm count (P=0.126) and the number of escalated-BEACOPP cycles received (P=0.261). None of these factors were found to contribute to the likelihood of spermatogenesis recovery.
Limitations, reasons for caution: Primary limitations are 1) the small sample-size, which limited statistical power and subgroup analysis, and 2) the retrospective nature, culminating in significant loss to follow-up and no schedule of analysis. Records also contained limited data on other possible risk factors for impaired spermatogenesis including radiotherapy, pre-treatment ESR, and bulky/mediastinal disease.
Wider implications of the findings: These results suggest that, while impaired spermatogenesis is initially universal after escalated-BEACOPP, spermatogenesis recovery is evidenced in a proportion of patients in the years following. This research will allow appropriate evidence-based risk-counselling of advanced-stage Hodgkin's lymphoma patients considering escalated-BEACOPP and facilitate more informed patient decision-making.
Trial registration number: Not applicable

P-057 Immunohistochemical Investigation of CatSper Ca2 + Channel Proteins in Normozoospermia and OAT patient groups in Progesterone Applied Culture Media.

N. Köprülü[1], T. Irez[2]

[1]Biruni University, Institute of Health Sciences- Department of Clinical Embryology, Istanbul, Turkey ;
[2]Istanbul Yeni Yuzyıl University, Medical Faculty- Clinical Embryology, İstanbul, Turkey

Study question: To investigate effect of progesterone, which is a strong chemoattractant candidate, on sperm tail motions that evaluate hyperactivation and CatSper expression in fertile and infertile cases.
Summary answer: Progesterone-treated sperms effected Catsper Channels and caused hyperactivation in infertile and fertile cases.
What is known already: Sperm chemotaxis causes intracellular calcium increase and hyperactivation motility as a result of Ca2 + flow into the cell with the activation of odor receptors. CatSper channels are cation channels necessary for fertility in rodents. Chemoattractants provide CatSper cation channel protein expression in the sperm principal peace region. Rabbit studies have shown that progesterone produces CatSper expression.
Study design, size, duration: Sperm chemotaxis causes intracellular calcium increase and hyperactivation motility as a result of Ca2 + flow into the cell with the activation of odor receptors. CatSper channels are cation channels necessary for fertility in rodents. Chemoattractants provide CatSper cation channel protein expression in the sperm principal peace region. Rabbit studies have shown that progesterone produces CatSper expression.
Participants/materials, setting, methods: After standard semen analysis, sperm prepared in two groups by the swim-up method was kept at 37 ° C for 45 minutes in culture medium containing progesterone. Motility and CatSper IHC were evaluated before and after incubation.
Main results and the role of chance: Progesterone was found to have important chemoattraction ability for hyperactivation effect on the sperm tail movement on normospermic and oligoasthenoteratospermic sperm that integrity in luteal phase doses. Infertile group showed less progressive motility, less hyperactivation and less CatSper expression than fertile Normospermia group. (p<0,05). Progesterone administration caused higher rate of hyperactivation and CatSper positivity in normospermic cases than OAT group (p <0.05).
Limitations, reasons for caution: We conducted our study in 56 cases. It is beneficial to conduct the study in larger groups.
Wider implications of the findings: The results of our study may be the pioneer of studies investigating the relationship between CatSper expression and sperm hyperactivation. A new research project is planned to examine IVF results in terms of infertility and unexplained infertility in men.
Trial registration number: 0

P-058 Combined treatment with myo-inositol and alpha-lipoic acid of sub-fertile men significantly improves semen competence in IVF cycles

F. Sozzi[1], P. Canepa[1], E. Maccarini[1], C. Massarotti[1], C. Rizzo[1], D.L. Alessandro[2], M. Gallo[2], E. Licata[2], P. Anserini[1], R. Rago[2], P. Scaruffi[1]

[1]IRCCS Ospedale Policlinico San Martino, UOS Fisiopatologia della Riproduzione Umana, Genova, Italy ;
[2]Ospedale Sandro Pertini - ASL Roma 2, UOSD Fisiopatologia della Riproduzione e Andrologia, Roma, Italy

Study question: Does combined treatment with myo-inositol and alpha-lipoic acid of sub-fertile patients improve semen competence in IVF cycles?
Summary answer: Sub-fertile men's diet oral supplementation with combined myo-inositol and alpha-lipoic acid improved sperm parameters and semen reproductive competence in IVF cycles.
What is known already: Human infertility affects 15% of couples in childbearing age, of which 40% is due to male factors. Alterations of sperm number, motility and morphology are mostly associated with male infertility. Some 30% to 80% of sub-fertile men may be associated to oxidative stress (OS) that damage spermatozoa. Furthermore, negative relations between OS and low success of IVF techniques has been observed. Antioxidant oral supplements are widely used in sub-fertile patients. We previously reported that a combination of nutraceuticals (myo-inositol, alpha-lipoic acid, folic acid, betaine, and vitamins) significantly improves sperm parameters in sub-fertile men.
Study design, size, duration: This pilot, multi-centric study was performed in 2018-2019 including 124 idiopathic, sub-fertile, 26-53 years aged men. Semen analysis was performed before (T0) and after 90 days (T90) of treatment and results were compared. A subset of 62 patients who underwent an IVF cycle at T0 with unexplained <70% fertilization rate (sperm count >1x10^6/ml, woman age <38 years, inseminated oocytes >6) took the antioxidant supplements. Outcomes of the paired T0 and T90 IVF cycles were compared.
Participants/materials, setting, methods: Patients' diet was supplemented with myo-inositol (1000 mg), alpha-lipoic acid (800 mg), coenzyme Q10 (200 mg), selenium (83 mcg), zinc (15 mg), vitamin B2 (2.8 mg), B6 (2.8 mg), B12 (5 mcg). Sperm analysis was assessed according to WHO manual. Semen OS was measured by MioxSIS® (Aytu BioScience). The IVF cycles were performed according to conventional procedures using fresh gametes of each couple. Wilcoxon and chi-square tests compared data before and after the antioxidant treatment.
Main results and the role of chance: Statistical analyses showed a significant increase of sperm concentration (P=0.0307) and motility (P=0.0003) after 90 days of treatment. After therapy with antioxidants it was observed a decreasing trend of OS levels in semen (normalized oxidation-reduction potential (mV/10^6 ml sperm). T0: 8.9+8.6; T90: 6.1+7.7). As regard as the outcomes of the IVF cycles, the fertilization rate was significantly higher in the T90 cycles with respect to T0 ones (P<0.0001). Moreover, results demonstrated a significant improvement in the embryo quality in the cycles performed at T90, in terms of higher embryo top quality rate (P=0.0001) and blastulation rate (P<0.0001). Any T0 IVF cycle did not result in a successful pregnancy. Conversely, in the T90 cycles we obtained 11/62 clinical pregnancies (30%) with an implantation rate of 19%.
Limitations, reasons for caution: These data will be confirmed in a larger sample size to correct primary endpoints for putative confounders. We also foresee to deeply investigate the effect of the antioxidant treatment on semen OS status and ultimately on sperm DNA integrity. Lastly, a prospective study will determine true clinical value and limitations.
Wider implications of the findings: The outcomes of this pilot study suggest that treatment of sub-fertile men with combined myo-inositol and alpha-lipoic acid may have beneficial effect on sperm quality and pregnancy rates. Therefore, such a "nutraceutical" diet supplementation may become an additional tool to reduce the semen OS, thus improving the success of IVF.
Trial registration number: not applicable

P-059 Improvement in mitochondrial integrity and fertility potential in men with Rheumatoid arthritis by yoga based lifestyle intervention

S. Gautam[1], R. Kumar[2], U. Kumar[3], R. Dada[1]

[1]All India Institute of Medical Sciences, Anatomy, New Delhi, India ;
[2]All India Institute of Medical Sciences, Urology, New Delhi, India ;
[3]All India Institute of Medical Sciences, Rheumatology, New Delhi, India

Study question: Can a yoga based lifestyle intervention bring about alterations in mitochondrial integrity and fertility potential in men with Rheumatoid arthritis?
Summary answer: Yoga not only reduces disease severity, minimizes usage of drugs but also increases mitochondrial copy number and improves mitochondrial integrity.
What is known already: The complex mechanism of Rheumatoid arthritis with infertility in men involves interactions between endocrine, immune, and reproductive systems. Association of autoimmunity with dysregulated androgen (hypogonadism) levels may cause transient infertility in men. Furthermore, usage of disease-modifying antirheumatic drugs (DMARDs) like cyclophosphamide, methotrexate, sulphasalazine etc. may result into decreased quantity and quality of sperm, mitochondrial dysfunctions, reduced fertility potential and ultimately permanent infertility. These drugs can cross blood-testis-barrier and induce changes in sperm impairing spermatogenesis. Complementary and alternative medicine like yoga reduces seminal oxidative stress and its consequences like DNA fragmentation in sperm nuclear/mitochondrial genome.
Study design, size, duration: Sixty males with RA were enrolled in this 12-week prospective, open-label, single-arm exploratory study, designed to explore the impact of yoga based lifestyle intervention (YBLI) on mitochondrial integrity and fertility potential in men with Rheumatoid arthritis.
Participants/materials, setting, methods: The participants were evaluated for pre (day 0) and post (12th week) levels of inflammatory markers: IL-6, IL-17A

and soluble HLA-G. Mitochondrial health was assessed by calculation of mitochondrial copy number variation and transcripts associated with mitochondrial integrity: *AMPK, PRC-1, TFAM, SIRT-1*. Sperm parameters as per WHO 2010 guidelines and reactive oxygen species (ROS), DNA fragmentation index (DFI), 8-hydroxy-2'-deoxy guanosine (8-OHdG) were estimated.
Main results and the role of chance: The mean levels of ROS ($p<0.05$***), DFI ($p<0.05$**) and 8-OH2dG ($p<0.05$**) levels were significantly reduced after 12 weeks of yoga intervention. We observed reduction in mean levels of CRP ($p<0.05$***), IL-6 ($p<0.05$**), IL-17A ($p<0.05$**) and soluble HLA-G ($p<0.05$**) at 12 weeks compared to baseline level (day 0). There was a significant increase in mitochondrial copy number and increased expression of transcripts that maintain mitochondrial integrity after 12 weeks with respect to the baseline levels (day 0).
Limitations, reasons for caution: Compliance of patients for Yoga based lifestyle intervention was poor, hence we enrolled large number of patients to achieve the desirable sample size.
Wider implications of the findings: Adoption of yoga based lifestyle intervention as an integral part of our lifestyle may hold the key to increase mitochondrial copy number, increase the expression levels of transcripts that maintain mitochondrial integrity and its associated consequences on physical, mental and reproductive health.
Trial registration number: N/A

P-060 TDG involves in the key maintenance pathways of neonatal spermatogonial cells

T.L. Lee[1]

[1]*The Chinese University of Hong Kong, School of Biomedical Sciences, Sha Tin, Hong Kong*

Study question: The role of TDG in spermatogonial cell development during neonatal stage
Summary answer: TDG involves in key pathways associated with spermatogonial cell maintenance at neonatal stage.
What is known already: Active DNA demethylation through 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) by TDG is essential for mouse embryonic and stem cell development, but its role remains elusive in SSC biology. Therefore, we examined the functional role of TDG in male germ cell development using our recent established germline-specific TDG knockout animal.
Study design, size, duration: 6-day old balb/c mouse with TDG KO (KO) were compared against the wild type and hemizygous controls.
Participants/materials, setting, methods: 6-day old balb/c mouse strain was used. Isolation of neonatal spermatogonial population was performed by OCT4 GFP signal.
Main results and the role of chance: Using KO TDG animal model, we found that TDG knockout males expressed a progressive loss of fertility. Transcriptome analysis by RNA-seq revealed altered gene expression of a subset of retinoic acid target genes, including Stra8 and Cyp26a1. Furthermore, we found that the demethylation of Stra8 during the KIT transition was impaired after depletion of TDG in Oct4-GFP+ undifferentiated spermatogonia. These results indicate that TDGmediated active demethylation regulates the differentiation of undifferentiated spermatogonia through the coupling of the retinoic acid signaling pathway.
Limitations, reasons for caution: The role of TDG in adult SSC stage was not studied.
Wider implications of the findings: A key question in SSC biology is whether and how epigenetic programs contribute to the cellular identity of SSC and specific gene expression programs. The study provides details about the molecular regulation of TDG in neonatal spermatogonial cell development and will allow the development of future male infertility treatment.
Trial registration number: not applicable

P-061 The presence of high risk human papilloma viruses in semen affects seminal parameters and sperm DNA quality in infertile men

E. Mastora[1], **A. Zikopoulos**[1], **E. Hatzi**[1], **I. Mpoumpa**[1], **I. Georgiou**[1], **K. Zikopoulos**[1]

[1]*University of Ioannina, Department of Medicine, Ioannina, Greece*

Study question: The aim of the present study was to assess the impact of high risk HPV infection on semen parameters and sperm DNA fragmentation index in infertile men.
Summary answer: High risk HPV seminal infection is probably responsible of lower sperm motility and higher DNA fragmentation index (DFI).
What is known already: HPV infection is a common sexually transmitted disease, related to genital warts and cancer. In males, human papilloma virus DNA has been found in the seminal fluids. HPV is commonly present in sperm samples. However, whether the prevalence of high risk HPV in semen is associated with modified semen parameters and sperm DNA fragmentation index has yet to be elucidated.
Study design, size, duration: From January to December 2018, about 505 infertile couples were analysed at the Human Reproduction Unit of Ioannina University Hospital. A total of 335 clinical and laboratory data from male partners were included in the study. Semen parameters and sperm DNA fragmentation index were compared among those with or without HPV in spermatozoa.
Participants/materials, setting, methods: A real time polymerase chain reaction assay was performed to detect the presence of HPV. All patients underwent a semen analysis. The amount of DNA fragmented sperm, expressed in DFI, was valuated using the sperm chromatin dispersion test. Semen parameters and sperm DNA fragmentation index were compared among high risk HPV positive and HPV negative men.
Main results and the role of chance: 49 (14,6%) of the total semen samples were HPV positive. Overall, 38/335 (11,3%) and 11/335 (3,3%) patients had high risk HPV and low risk HPV, respectively. HPV31 was the prevalent type. The amount of DNA fragmented sperm between high risk HPV positive and negative males was different. Sperm motility was lower (P=0.007) and SDF values were higher (P=0.003) in infected men compared to those without HPV.
Limitations, reasons for caution: Main limitation is the relatively small sample size.
Wider implications of the findings: The results suggest that high risk HPV infection probably impairs sperm DNA quality. These observations point out the importance of testing seminal HPV presence in everyday clinical practice.
Trial registration number: None

P-062 Permanent testicular metabolomic and lipidomic signatures induced by the adoption of a high-fat diet in childhood and sperm quality in adulthood

L. Crisostomo[1], **L. Rato**[2], **I. Jarak**[1], **R. Videira**[1], **K. Starcevic**[3], **T. Masek**[3], **J. Raposo**[4], **O. Pedro**[5], **M.G. Alves**[1]

[1]*Institute of Biomedical Sciences Abel Salazar- University of Porto, Laboratory of Cell Biology- Department of Microscopy, Porto, Portugal ;*
[2]*University of Beira Interior, Faculty of Health Sciences, Covilhã, Portugal ;*
[3]*University of Zagreb, Veterinary Medicine, Zagreb, Croatia ;*
[4]*Nova University Lisbon, Public Health Department, Lisbon, Portugal ;*
[5]*QOPNA & LAQV- University of Aveiro, Chemistry Department, Aveiro, Portugal*

Study question: Can a diet intervention in young mice fed high fat diet improve sperm quality in adulthood?
Summary answer: The adoption of high-fat diets from childhood to early adulthood causes irreversible metabolic and lipidomic changes in testis, reflected in poorer sperm quality.
What is known already: The prevalence of overweight and obesity has soared worldwide in recent decades, fueled by poor dietary habits as the adoption of high-fat diets (HFD). Besides, the adoption of HFD often occurs in childhood, triggering the onset for these conditions and associated comorbidities, such as diabetes, at increasingly younger age. Thus, men can spend most of their sexual development suffering from a metabolic disease, with unknown consequences for future sperm quality. A change in diet habits may prevent the progression of the disease, but it is not known whether it can rescue potential negative effects of HFD to male fertility.
Study design, size, duration: Hereby, we used a rodent model to study diet intervention during early adulthood, after childhood obesity, to correct HFD and how it affects the metabolic and reproductive health later in life. For that, after weaning (day 21 after birth), 3 groups of 12 mice each were fed with different diet regimen during 200 days: CTRL – standard mucedola; HFD – high-fat diet; HFDt – high-fat diet for 60 days, then replaced by standard mucedola.

Participants/materials, setting, methods: Animals were then sacrificed, and tissues collected and weighed. Epididymal sperm parameters (sperm counts, motility, viability and morphology) were evaluated. Animals biometric, metabolic (fasting glucose, ipGTT, ipITT, HOMA2) and endocrine (serum insulin, testosterone, estrogen, gonadotrophins) parameters were studied. Testicular metabolites were extracted and characterized by 1H-NMR and LC-MS. Testicular mitochondrial (citrate synthase, complex I, II and IV) and antioxidant activity (CAT, SOD, GSR, GPx) were also evaluated by colorimetric and fluorometric methods.

Main results and the role of chance: Our results showed an induction of a pre-diabetic state due to consumption of a HFD. Based on HOMA2, diet intervention in early adulthood prevents the development of metabolic syndrome in adulthood, although insulin resistance is maintained. No changes were observed in endocrine function. Importantly, sperm parameters were not recovered after diet intervention. Spermatozoa motility and viability were still affected in mice fed HFD during childhood, even after a dietary intervention, particularly head defects. HFD, even if transient, promoted changes in testicular metabolome and lipidome. Notably, it affected the glutamate, pyruvate, ethanol and ammonia metabolism, and promoted the storage of unsaturated fats. Interestingly, no differences were found in mitochondrial and antioxidant enzyme activities. The progression of metabolic syndrome due to HFD was found to be correlated with the changes in testicular metabolism. In turn, testicular metabolic remodeling was strongly correlated with sperm parameters. Therefore, a causal relation between the adoption of HFD in childhood and poorer sperm quality in adulthood is not just a stochastic event.

Limitations, reasons for caution: Despite using this mouse model, we are unable to state whether normal sperm quality could be achieved even later in life, or if the adoption of HFD in adulthood triggers the same deleterious effects in sperm parameters.

Wider implications of the findings: Diet intervention reverses some of the detected changes but cannot overcome the long-term consequences of HFD to sperm quality. Our conclusions must raise awareness about tackling childhood obesity, to avoid irreversible damage for the reproductive health of the future fathers, with unpredicted effects to their progeny.

Trial registration number: The authors declare no conflict of interest. this work was supported by the Portuguese Foundation for Science and Technology: L. Crisóstomo (SFRH/BD/128584/2017), M.G. Alves (IFCT2015 and PTDC/MEC-AND/28691/2017), QOPNA (UID/QUI/00062/2019) and UMIB (UID/Multi/00215/2019) co-funded by FEDER funds (POCI/COMPETE 2020); by the Portuguese Society of Diabetology: L. Crisóstomo and M.G. Alves ("Nuno Castel-Branco" research grant); and by the Amadeu Dias Foundation: M.G. Alves and P.F. Oliveira.

P-063 Blood plasma miR-20a-5p expression as a potential non-invasive diagnostic biomarker in patients with non-obstructive azoospermia

G. Cito[1], P. Pinzani[2], R. Fucci[3], R. Picone[3], F. Salvianti[2], C. Giachini[3], P. Falcone[3], E. Micelli[3], A. Cocci[1], P. Verrienti[1], A. Minervini[1], M. Carini[1], A. Natali[1], M.E. Coccia[3]

[1]Careggi Hospital- University of Florence, Dept. of Urology, Florence, Italy ;
[2]Careggi Hospital- University of Florence, Molecular and Clinical Biochemistry Unit, Florence, Italy ;
[3]Careggi Hospital- University of Florence, ARTs Center, Florence, Italy

Study question: To evaluate the blood plasma miR-20a-5p expression in infertile patients with non-obstructive azoospermia (NOA) compared to healthy normozoospermic men.

Summary answer: The overexpression of blood plasma miR-20a-5p seems to be directly related to damaged spermatogenesis, including conditions of NOA.

What is known already: Recently, alterations in the expression of specific microRNAs (miRNAs) in semen have been linked to altered spermatogenesis suggesting their expression could be used as potential infertility biomarkers and explain the molecular mechanisms underlying the altered spermatogenesis. In previous animal studies dysregulation of miR-20a-5p was found to be under-expressed in the low motile sperm and potentially target genes associated with cell apoptosis and spermatogenesis alteration. Considering previous findings with miRNAs in human plasma, serum and urine, we believe that the expression of miR-20a-5p might represent a new source of non-invasive diagnostic biomarkers for idiopathic male infertility.

Study design, size, duration: From January 2018 to December 2019, 24 infertile couples who referred to our ARTs Centre were prospectively enrolled. All the patients were included into two groups: Group 1 comprised infertile men with NOA, Group 2 involved healthy normozoospermic men belonging to couples with female infertility tubal factor who achieved pregnancy using IVF or ICSI.

Participants/materials, setting, methods: The expression of circulating miR-20a-5p was assessed by RT qPCR in plasma samples. A relative quantification strategy was adopted using the $2^{-\Delta Cq}$method to calculate the target miR-20a-5p expression with respect to miR-16-5p as an endogenous control. Total cell-free RNA extracted from 0.5 ml plasma using the mirVana PARIS kit (ThermoFisher Scientific, USA) was submitted to RT-qPCR using TaqMan Advanced miRNA cDNA Synthesis Kit and TaqMan® Advanced miRNA Assays (ThermoFisher Scientific) according to the manufacturer's instructions.

Main results and the role of chance: Group 1 included 14 patients, Group 2 comprised 10 men. No genetic abnormalities and significant comorbidities were detected in all the patients enrolled. Mean male age was 35.6 ± 4.2 years. Considering the Group 1, mean FSH value was 19.4 ± 7.8 IU/l, mean LH value was 8.5 ± 3.4 IU/l, mean TT was 12.5 ± 3.9 nmol/l, mean TSH was 2.0 ± 1.1 mIU/l and mean PRL was 10.5 ± 3.2 ng/ml. Mean right testicular volume was 8.9 ± 5.2 ml, mean left testicular volume was 8.2 ± 4.5 ml. All NOA patients underwent testicular sperm extraction (TESE). Successful sperm retrieval (SR) with cryopreservation was found in 8/14 patients (overall SR rate: 57.1%). Mean sperm concentration was 0.001 ± 0.0001 x 10^6/ ml. Mean non progressive motility was 0.2 ± 0.6 %. Mean number of biosystem straws collected was 3.2 ± 2.0. All healthy normozoospermic men showed serum hormonal levels and testicular volume in the normal range. Mean miR-20a-5p value was 0.25 ± 0.20 and 0.06 ± 0.02 in the Group 1 and Group 2, respectively. Thus, the relative expression of miR-20a-5p was significantly higher in patients affected by NOA than in healthy normozoospermic control subjects (p=0,026).

Limitations, reasons for caution: Further larger studies are needed to evaluate the correlation between this marker and testicular histopathological findings in order to increase its usefulness as a predictor in the clinical practice.

Wider implications of the findings: Blood plasma miR-20a-5p could represent a potential non-invasive diagnostic biomarker in infertile patients with non-obstructive azoospermia who undergo TESE. A possible correlation of this marker with testicular histopathological findings could allow the clinician to correctly counsel the azoospermic patients in performing surgery for fertility purpose.

Trial registration number: not applicable

P-064 A new modified method: Aniline Blue (AB) stain in live spermatozoa reveals that all motile sperms not contain histones. (Preliminary study. EcoFoodFertility Project).

"Abstract withdrawn by the authors"

P-065 Novel PMFBP1, TSGA10 and SUN5 variants in acephalic spermatozoa syndrome and the overcoming this male infertility

L. Weina[1], W. Ni[2], Z. Huan[3], Y. Silu[1], L. Ge[3], L. Guangxiu[3], L. Gang[1]

[1]Reproductive and Genetic Hospital of CITIC-Xiangya, Andrology Laboratory, Changsha, China ;
[2]Central South University, Institute of Reproduction and Stem Cell Engineering, Changsha, China ;
[3]Reproductive and Genetic Hospital of CITIC-Xiangya, Reproductive center, Changsha, China

Study question: What are the genetic diagnosis and effective therapeutic strategies to overcome infertility in acephalic spermatozoa syndrome?

Summary answer: We identified four novel mutations in *PMFBP1*, *TSGA10*, and *SUN5* in three out of eleven patients. These male infertility could be overcome by ICSI.

What is known already: Acephalic spermatozoa are a rare teratozoospermia which leads to male infertility. The vast majority of acephalic spermatozoa cases are idiopathic, and 52.63% (51.22%, 21/41 SUN51-5; 50%, 7/14 PMFBP16 7; 100%, 1/1 TSGA108; 100%, 1/1 BRDT9) have been associated with definitive genetic etiology. Considering that negative/negative results are rarely published,

the previous study population mainly covers Hefei and Xiamen, and the contribution of these genes to acephalic spermatozoa might not be as high as described previously.

Study design, size, duration: This genetic study used whole-exome and Sanger sequencing to discover and identify related-genes mutations, and was followed by a series of studies to certificate potentially damaging.

Participants/materials, setting, methods: 11 patients with acephalic spermatozoa were recruited from our hospital in China in the first half of year 2019. Genomic DNA samples from the individuals were extracted from peripheral blood. All samples were subjected to whole-exome sequencing and variant filtering, using Sanger sequencing to identify mutations. Identified mutations were further investigated using bioinformatics, papanicolaou staining, CASA, transmission electron microscopy(TEM), scanning electron microscopy (SEM) and RT-PCR.We evaluated their available clinical data and pregnancy outcomes.

Main results and the role of chance: We identified one compound heterozygous variant (c.361C>T[p.Gln121Ter],c.2089-1G>T) in *PMFBP1*, one homozygous mutation (c.1739A>C[p.Gln580Pro]) in *TSGA10*, and one homozygous variant (c.772C>T[p.Arg258Cys]) in *SUN5* in three unrelated infertile men.The remaining were new gene results that should be further validated by animal models. RT-PCR in blood revealed that mutation causes loss of the wild-type splice site, which leads to aberrant splicing of *PMFBP1* mRNA and consecutive formation of the *PMFBP1* alternative transcript (p.Ile697Leufs*257). All of these mutations cause protein truncations or lead to point mutations and have a recessive inheritance pattern. All patients with acephalic spermatozoa had different phenotypes and proportions caused by their different ultrastructure. Patients with p.Arg258Cys in *SUN5* showed 70% acephalic spermatozoa and 25% microcephalic spermatozoa but no so-called round-headed like spermatozoon,which suggest that a variant in *SUN5* does not result in microcephalic spermatozoa in patient 3. There are other pathogenic genes for his multiple malformations in spermatozoa, which need to be further explored. *PMFBP1*, *TSGA10* and *SUN5* may associate with the centrosome and basal body and participate in completing the coupling apparatus, ultimately resulting in acephalic spermatozoa but not delayed or stagnated embryonic development. *PMFBP1*, *TSGA10* and *SUN5* mutation-associated male infertility could be successfully overcome by ICSI in humans.

Limitations, reasons for caution: Our pilot study was intended as proof of principle, and the number of patients is low. Although these patient defects could be resolved by microinjection of the tailless sperm head into the oocyte, further studies are needed to validate the safety of offsprings.

Wider implications of the findings: We summarized known data on the genetic contribution of genes to acephalic spermatozoa, expanded the mutation spectrum, and analyzed the variety of phenotypes for each type of mutation. This work lays the foundation for the genetic diagnosis, genetic counseling and treatment of acephalic spermatozoa syndrome in China,for reproductive options.

Trial registration number: Not applicable.

P-066 Effect of high fat diet induced paternal obesity and micronutrient intervention on offspring testicular antioxidant capacity

M.M. Billah[1], S. Khatiwada[1], V. Lecomte[1], M.J. Morris[1], C.A. Maloney[1]

[1]UNSW Sydney, School of Medical Sciences, Sydney, Australia

Study question: Does paternal obesity perturb testicular antioxidant capacity in father and offspring? If so, can we mitigate this transgenerational effect by micronutrient intervention in father?

Summary answer: Obesity-related reductions in testicular SOD and glutathione peroxidase activity in fathers and increased SOD activity in offspring were ameliorated by micronutrient intervention in obese fathers.

What is known already: Obesity has tripled in males of reproductive age since 1970. As well as affecting individuals, obese people can produce offspring that are predisposed to metabolic and reproductive complications. One possible mechanism is oxidative stress which increases sperm DNA damage, alters sperm epigenetic profile thus affecting future generations. It is known that obesity is associated with higher levels of oxidative stress. We sought to reduce this effect by providing a micronutrient supplement (consisting of metabolites of one-carbon metabolism) targeting oxidative stress to prospective rat fathers.

Study design, size, duration: Founder (F0) male Sprague Dawley rats (12 per group, 48 total) were weaned (Day21) onto control (C) or HFD (H), or micronutrient supplemented versions of these (CS; HS). At 19 weeks of age, they were mated with CD females. 48 F1 offspring (one from each F0 pairing) were weaned onto chow diet, generating four F1 groups. At cull (30 weeks for F0, 23 weeks for F1), right testis was harvested and stored at -80°.

Participants/materials, setting, methods: Testis was assayed for the level of lipid peroxidation (MDA), glutathione and the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) in F0 founders and MDA, SOD, CAT in F1 offspring according to kit protocols. Susceptibility index of prooxidative damage was calculated in F0 testis from the ratio SOD:CAT+GPx. Statistical analyses were performed using IBM SPSS v23.0 software. Data were presented as mean±SEM and analysed by two-way ANOVA followed by post hoc LSD.

Main results and the role of chance: In founders, HFD significantly increased body weight (H: 765.1±16.5 vs C: 632.5±15.1 g; p= 0.0001) which was normalized by supplementation (HS: 601.9±11.8 g vs H; p=0.0001). Supplemented HFD fed fathers had reduced testicular lipid peroxidation (HS: 7.5±2.1 vs H: 12.8±1.7 nmol/g testis; p=0.01). No significant effect of HFD was observed in testicular lipid peroxidation. HFD fed fathers had reduced testicular enzymatic antioxidant activity (SOD & GPx: H vs C; p=0.002) while supplementation restored these activities (HS vs H; p=0.002). Moreover, supplementation increased a major cellular antioxidant, glutathione (HS: 4179.7±197.1 vs H: 3421.2±194.8 nmol/g testis; p=0.004). No significant effect of HFD was observed on testicular glutathione levels. Susceptibility index of prooxidative damage was significantly increased by chronic HFD intake (H: 1.7±0.2 vs C: 1.1±0.1; p=0.002) and normalized by supplementation (HS: 1.2±0.1 vs H; p=0.004). No effect of HFD and supplementation was observed in F0 testicular catalase activity. In F1 testis, offspring from HFD fed fathers had increased testicular SOD activity (H: 67.2±6.5 vs C: 49.6±6.1 U/mg protein; p=0.04) whereas, offspring from HS fathers had decreased SOD activity (HS vs H; p=0.02). No effects of paternal diet and supplementation were observed in offspring testicular lipid peroxidation level and catalase activity.

Limitations, reasons for caution: F0 testis was harvested at 30 weeks rather than at mating (19 weeks). As we have no measurement of sperm DNA damage in F0 and F1 males, it is difficult to comment on underlying mechanisms of transgenerational effects of paternal obesity at this stage.

Wider implications of the findings: HFD induced paternal obesity may affect offspring testicular antioxidant capacity which might relate to metabolic and reproductive complications in future generations. Our micronutrient supplementation might be a novel intervention to restore antioxidant capacity in obese father and their offspring. Supplementation of non-obese individual may have deleterious effects in next generation.

Trial registration number: Not applicable

P-067 Seminal oxidative stress and sperm DNA fragmentation in men from couples with idiopathic recurrent pregnancy loss or infertility

J.M. Kold[1], M.R. Dalgaard[1], F. Dardmeh[2], H. Alipour[2], O.B. Christiansen[1]

[1]Aalborg University Hospital, Department of Obstetrics and Gynaecology, Aalborg, Denmark ;

[2]Aalborg University, Department of Health Science and Technology, Aalborg, Denmark

Study question: Are seminal oxidative stress (OS) and sperm DNA fragmentation (SDF) correlated, and can they explain idiopathic infertility or recurrent pregnancy loss (RPL)?

Summary answer: OS and SDF levels are not different between case groups and fertile controls and OS correlates to SDF in infertile but not in RPL cases.

What is known already: Around 15% of all couples experience infertility while 1-2% experience RPL. Approximately 25% of infertility and 40% of RPL cases are considered idiopathic. Studies have reported that seminal OS and SDF appear frequently in men from couples with infertility or RPL. Furthermore, the use of sperm with a high level of SDF has been associated with poor artificial reproductive technology outcome. However, there is no consensus on the impact of and whether to test for seminal OS or SDF in cases of idiopathic RPL or infertility.

Study design, size, duration: This clinical case-control study aimed to include 30 men in each of the two case groups and 30 fertile controls. The data collection and assessments were according to the protocol planned between June 2019 and July 2020 at a tertiary university centre for infertility and RPL treatment.
Participants/materials, setting, methods: Semen samples from male partners of couples with idiopathic infertility (n=23), idiopathic RPL without concomitant infertility (n=20), and fertile men (n=29) were assessed for SDF using sperm chromatin dispersion test, concentration, motility and morphology by the Sperm Class Analyzer (Microptic S.L., Spain) computer aided sperm analysis (CASA) system. Seminal OS was measured as static oxidation-reduction potential (sORP) using Male Infertility Oxidative System (MiOXSYS, Aytu BioScience Inc, USA). sORP were normalised to semen concentration.
Main results and the role of chance: The infertile and RPL groups, showed no significant difference in the levels of OS (p=0.59 and p=0.67, respectively) or SDF (p=0.85 and p=0.11 respectively) when compared to fertile controls. There was a significant correlation between OS and SDF in the infertile group (R=0.404, p=0.028) but not in RPL group (R=0.225, p=0.18). Additionally, in the infertile group 84.6 % of men with a high level of OS (normed sORP >1.38mV/106 sperm mL) had a high level of SDF (> 15 %), whereas 60 % with low OS had a low level of SDF. The infertile men were from couples diagnosed with idiopathic infertility based on traditional (manual) semen analysis, but nevertheless analysed by CASA 78.3% had a motility below the World Health Organisation (WHO) reference values.
Limitations, reasons for caution: Several of the men diagnosed with idiopathic infertility had a motility below the WHO reference values. Thus, this study had not only examined men from couples with idiopathic infertility as intended but also men who may have occult male factor infertility.
Wider implications of the findings: OS determined as normed sORP may be useful to evaluate the risk of SDF in men with infertility. Several men diagnosed with idiopathic infertility had low motility when samples were assessed using CASA, indicating that these men had been misdiagnosed with idiopathic infertility instead of male factor infertility.
Trial registration number: Ethical committee approval no. N-20190023

P-068 A new oral treatment based on the ATM/ATR DNA repair pathways enhancement reduce sperm DNA damage and improve semen parameters

J. Franco[1], S. Lara-Cerrillo[2], Á. Vives-Suñé[3], M. Ballester-Ferrer[3], T. Lacruz-Ruiz[2], C. Rosado-Iglesias[2], M. Velasco-Álvarez[4], E. Rodríguez-Ferradas[4], A. Jiménez-Macedo[5], J. Carrera-Rotllan[5], A. García-Peiró[2]

[1]Hospital Ruber Internacional, Human Reproduction, Madrid, Spain ;
[2]CIMAB- Barcelona Male Infertility Centre, Research & Development, Sant Quirze del Vallés - Barcelona, Spain ;
[3]Hostpital Universitari Dexeus, Reproducció Assistida, Barcelona, Spain ;
[4]Instituto Vasco de Fertilidad - Grupo IVF Life, Reproducción asistida, San Sebastián, Spain ;
[5]Unitat d'Endocrinologia Ginecològica, Reproducció assistida, Vic, Spain

Study question: Can double-strand sperm DNA breaks and semen parameters be improved by oral administration of antioxidants and ATM/ATR-enhancers?
Summary answer: A new treatment based on ATM/ATR-repair-pathways enhancement significantly reduced double-strand sperm DNA breaks and improved semen parameters.
What is known already: Double-strand breaks (DSB) take place during male meiosis to enable DNA recombination and packaging. These breaks must be repaired later to ensure sperm genome integrity. However, defective chromatin condensation, incomplete apoptosis and oxidative stress can increase both DSB and single-strand breaks (SSB). Several studies demonstrated that DSB are a major source of chromosome abnormalities causing miscarriage. The ATM/ATR kinases play an essential role in genomic maintenance through the repair of DSB and the activation of cell-cycle checkpoints. Moreover, seminal parameters are improved by oral treatments including antioxidants. The enhancement of ATM/ATR-repair-pathways could be a new strategy to improve sperm quality.
Study design, size, duration: This prospective and multicentric study included 71 infertile patients that were randomly divided into three groups, receiving a different treatment: Control group (Androferti, n = 21); Experimental formula 1 (high concentration of ATM/ATR-enhancer, n = 25); Experimental formula 2 (low concentration of ATM/ATR-enhancer, n = 25). Semen parameters and sperm DNA fragmentation were determined before and after 13 weeks of treatments.
Participants/materials, setting, methods: All patients included presented high values of DSB. Patients receiving medication or other fertility treatments before the start of the study were excluded. DSB and SSB were analysed through the Neutral and Alkaline CometFertility assay (CIMAB, Spain), respectively. Semen parameters including concentration, total sperm count and progressive motility were determined according to the last World Health Organization guideline.
Main results and the role of chance: DSB values were significantly reduced by all three treatments (p=0.042). The greatest reduction was observed in Formula 1, which reduced 27.1%. Formula 2 and the Control group reduced 19.9% and 15.8%, respectively. SSB was reduced by all three treatments. Formula 1 significantly reduced SSB 19.5% (p=0.040). Even not statistically significant, Formula 2 and the Control group reduced SSB 6.8% and 8.6%, respectively.
Regarding seminal parameters, Formulas 1 and 2 increased sperm concentration 2.11% (p=0.046) and 53.55% (p=0.200) respectively. The Control treatment reduced 20.14% this parameter (p=0.160). Total sperm count was improved by experimental treatments with an increase of 17.41% (p=0.135) and 113.13% (p=0.012) for Formulas 1 and 2, respectively. The Control group reduced 23.64% this parameter (p=0.001). Formulas 1 and 2 showed a moderate improvement in progressive sperm motility (9.10% and 1.08%, respectively) despite not being statistically significant (p=0.072 and 0.067, respectively). Not significantly, the Control treatment reduced 14.14% the progressive motility (p=0.200).

None of the experimental ATM/ATR-enhancement-based treatments showed any adverse effects on sperm genome integrity or other semen parameters. Formula 1 proved to be the most effective treatment to reduce sperm DNA fragmentation. On the other hand, Formula 2 proved to be the best treatment improving seminal parameters.
Limitations, reasons for caution: Patients compliance with treatment cannot be guaranteed. For this reason, patients were excluded from the study when there was a suspicion that the intake of the treatment did not follow the protocol instructions.
Wider implications of the findings: DSB are a major cause of chromosomal abnormalities in a zygote, cause a delay in embryo development and impair embryo implantation. Prevention of sperm DNA fragmentation through effective oral treatments focused on improving DNA damage repair and/or activating apoptosis systems would be of great interest to patients affected by DSB.
Trial registration number: Does not apply.

P-069 Association of serum metabolomic profile by nuclear magnetic resonance spectroscopy with semen parameters.

A. Taylor[1], K. Al Rashid[2], M.A. Lumsden[2], N. Goulding[1], D. Lawlor[1], S. Nelson[2]

[1]UNiversity of Bristol, Bristol Medical School, Bristol, United Kingdom ;
[2]Universiy of Glasgow, School of MEdicine, Glasgow, United Kingdom

Study question: To assess the association of 155 circulating metabolic measures relevant to lifestyle and metabolic health with sperm parameters (as measured by concentration, motility and total motile sperm count (TMSC)).
Summary answer: An extensive range of metabolites were not associated with semen parameters, highlighting the potential limited contribution of lifestyle modification to improve sperm count or motility.
What is known already: Several observational studies and small randomised controlled trials (RCTs) have investigated the association between metabolic health, nutritional supplements and lifestyle modification on sperm parameters with some, but not all studies, suggesting that improved metabolic health may be associated with improved semen parameters. However, these studies have been limited to a range of metabolic measures with limited adjustment for confounders, and recent large well-conducted RCTs of supplementation have not observed an improvement in sperm parameters.
Study design, size, duration: Cross-sectional study of 325 men prospectively recruited between 1 April 2017 and 31 March 2019.
Participants/materials, setting, methods: Men intending to undergo assisted conception at a University Hospital, had a detailed demographic, lifestyle, fertility and medical history and semen analysis. Non-fasting serum lipids, lipoprotein subclasses, and low-molecular weight metabolites (including amino

acids, glycolysis and inflammatory markers) were quantified by NMR spectroscopy. Multivariable linear and logistic regression was used to examine the associations of serum metabolic profiles (exposures), with functional sperm concentration, motility and TMSC (outcomes) with adjustment for confounders.
Main results and the role of chance: Participants were mean 37.2 (SD 5.7) years and had a median sperm concentration of 35 million/ml (IQR 15, 69 million/ml) and median motility of 53% (IQR 42,67). 76% of men had a TMSC >15 Million, 10% 5-15 Million and 14% <5 Million. In both univariate and adjusted analyses an extensive range of lipids and lipoproteins, acetate, beta-hydroxybutyrate, creatinine, albumin, glucose and the amino acids; alanine, glutamine, glycine, histidine, isoleucine, leucine, valine, phenylalanine and tyrosine did not show strong statistical evidence of associated with sperm concentration, motility, or the odds of having a reduced or low TMSC (all $_P_{Bonferroni} > 0.0029$).
Limitations, reasons for caution: Residual confounding may have resulted from crude questionnaire measurements, though we cannot think of masking confounders. Sperm parameters were measured on a single sample, in accordance with recent WHO guidance, and by non-automated techniques which may have introduced random measurement error that could have attenuated to the null.
Wider implications of the findings: We did not find any robust associations between a range of established and novel metabolic measures with semen parameters. Replication of our observed null results is critical given current interest in improving semen parameters through lifestyle modification.
Trial registration number: Not applicable

P-070 The effect of sperm DNA fragmentation index on the outcome of intrauterine insemination

D. Gunasheela[1], R. Nayak[1], B.S. Indrani[1], L. Sasikala[1], P. Ajitha[1], W.Y. Son[2]

[1]Gunasheela Surgical & Maternity Hospital, Obst & Gyne, Bangalore, India ; [2]McGill University Health Centre MUHC- Royal Victoria Hospital- Canada, Embryology, Montreal, Canada

Study question: Can sperm DNA fragmentation affect reproductive outcomes of intrauterine insemination?
Summary answer: High sperm DNA fragmentation (DFI: > 30%) is associated with poor reproductive outcomes in couples undergoing IUI.
What is known already: The pregnancy outcome after IUI procedures was unpredictable because several possible factors were involved in the process. A number of studies concerning the association between DFI and pregnancy outcome after IUI have been performed. Some studies showed that high DFI was positively correlated with low pregnancy rate after IUI. However, several other reports have found that DFI is not correlated with pregnancy outcome in IUI. The predictive value of DFI in reproductive outcome of IUI remains controversial.
Study design, size, duration: This prospective observational study included 413 women who underwent IUI cycle from October 2017 to October 2019. The male partners of the infertile couple underwent sperm-DFI by TUNEL or SCD assay. Depending on DFI, they were divided into three groups; Group 1 (DFI-negative:<15%), Group 2 (DFI-fair positive:15-30%) and Group 3 (DFI-high positive: > 30%). The clinical & ongoing/live birth rates were compared among the three groups. P < 0.05 was considered statistically significant
Participants/materials, setting, methods: This study was conducted in infertile couples attending our ART center, who planned for IUI and tested DFI ((n= 413), with also signed the consent form. The exclusion criteria were men not consenting to undergo the DFI test, those with oligozoospermia (< 15 million/ml) and cases of donor semen. Ovulation induction in IUI cycles was performed by clomiphene citrate 50–100 mg or Letrozole 5 mg with or without human menopausal gonadotropin (hMG), or hMG alone.
Main results and the role of chance: Out of 413 men who had tested DFI, 80 (19.3 %), 247 (59.8%) and 86 (20.8%) men were Group 1, 2 and 3, respectively. The clinical pregnancy and ongoing/live rates in Group 3 (11.6%, 10/86; 8.1%, 7/86) was significantly lower than those of Group 1 (17. 5% 14/80; 15.0%, 12/80) and Group 2 (16.6%, 41/247; 14.2%, 35/247) ($P < 0.05$). However, there was no statistically significantly difference in the reproductive potential between Group 1 and Group 2.
Limitations, reasons for caution: Sample size is still low even after collecting data for 2 years and more patients are required to better address this issue**.**
Wider implications of the findings: Sperm DNA fragmentation may be a potential predictor of reproductive outcomes of IUI.
Trial registration number: Not applicable

P-071 The impact of physical activity on semen analysis parameters in healthy males undergoing fertility investigation

N. Vlahos[1], A. Philippou[2], E. Maziotis[1], E. Alexopoulou[1], P. Giannelou[2], A. Rapani[1], S. Grigoriadis[1], A. Gryparis[1], P. Bakas[1], C. Siristatidis[1], D. Tzanakaki[1], M. Koutsilieris[2], M. Simopoulou[1]

[1]Aretaieio Hospital- National and Kapodistrian University of Athens, Second Department of Obstetrics and Gynecology, Athens, Greece ; [2]National and Kapodistrian University of Athens- Medical School, Physiology, Athens, Greece

Study question: Do levels of physical activity (PA) and metabolic equivalent (METs) correlate to parameters of semen analysis in healthy males?
Summary answer: Men reporting with moderate levels of PA present with higher sperm concentration, progressive and total motility, compared to men presenting with low levels of PA.
What is known already: The potential association between semen analysis parameters and environmental as well as lifestyle factors-including physical activity-has been extensively investigated in literature. Recent studies demonstrate that moderate physical activity may improve semen analysis parameters. However, discordant conclusions are reported on this topic. Furthermore, existing evidence is of poor quality as evident in recent systematic reviews and meta-analyses. This underlines the requirement for conducting large well-designed studies. The aim of this study was to investigate the possible effect of physical activity on semen parameters, evaluating the metabolic equivalent, in healthy males undergoing fertility investigation.
Study design, size, duration: A total of 223 men were recruited for this present prospective observational single-center study from November 2015 to October 2017. Participants were 18-40 years old, with a Body Mass Index (BMI) of 18.5-29.9 kg/m2. Men subjected to medication administration as part of a therapeutic regime, with current or previous diagnosis of cancer, endocrinological or genetic disorders, with a history of diseases that may affect the reproductive system and azoospermic men were excluded from the study.
Participants/materials, setting, methods: The participants were interviewed according to the Global Physical Activity Questionnaire (GPAQ). They were divided into 3 groups -namely, low, moderate and high PA- according to their physical activity levels as suggested by the World Health Organization (WHO). Semen analysis was performed according to WHO 2010 guidelines.
Main results and the role of chance: Forty men were categorized in the Low PA group, 117 in the moderate PA group and 66 in the high PA group. No statistically significant difference between the three groups was observed regarding the age, BMI, smoking status and the number of days of abstinence. No correlation was observed between the levels of METs and sperm concentration, total motility or the percentage of normal morphologically spermatozoa. The level of METs were negatively correlated with progressive motility (p=0.01). The Kruskal-Wallis test revealed differences between the groups regarding progressive (p=0.001) and total motility (p=0.04). In the post-hoc analysis, the moderate PA group presented with higher total motility (43.08±18.34 vs 36.43±16.01; p=0.01) and progressive motility (31.86±17.69 vs 25.75±16.50; p=0.04) when compared to the low PA group. The three groups-low, moderate and high PA-did not differ statistically significantly regarding neither sperm concentration (26.04±21.04 vs 55.53±37.16 vs 47.86±32.83; p=0.25), nor normal morphologically spermatozoa (12.35±6.69 vs 10.64±7.65 vs 10.82±7.13; p=0.80). The moderate PA group presented less risk for abnormal semen analysis compared to the low PA group (RR:0.65;95%CI:0.52-0.81;p=0.01). The high PA group presented with similar risk for abnormal semen analysis compared to either the low (RR:0.82;95%CI:0.66-1.03;p=0.10) or the moderate PA group (RR:1.26;95%CI:1.00-1.60;p=0.06), albeit marginally.
Limitations, reasons for caution: The present study corresponds to a single time-frame regarding semen analysis, as a second evaluation was not performed. Furthermore, the small sample size and the single-center nature of this study present as limitations.

Wider implications of the findings: The moderate PA group presented with a higher total and progressive motility, as well as with a lesser risk regarding abnormal semen analysis compared with the low PA group. To conclude, results presented herein demonstrate that moderate PA may improve semen quality, especially spermatozoa motility, buttressing existing published data.
Trial registration number: not applicable

P-072 Zona pellucida selects spermatozoa with good DNA fragmentation

R. Ganeva[1], D. Parvanov[1], D. Vaneva[2], M. Vasileva[3], G. Stamenov[4]

[1]Nadezhda Woman's Health Hospital, Research and development, Sofia, Bulgaria ; [2]Nadezhda Woman's Health Hospital, Andrology, Sofia, Bulgaria ; [3]Nadezhda Woman's Health Hospital, Embryology, Sofia, Bulgaria ; [4]Nadezhda Woman's Health Hospital, Obstetrics and gynecology, Sofia, Bulgaria

Study question: To evaluate and compare the sperm DNA fragmentation in normozoospermic patients after swim up preparation and zona pellucida binding sperm isolation.
Summary answer: Zona pellucida selected spermatozoa from normozoospermic patients have significantly lower DNA fragmentation than native semen and those prepared only by swim up.
What is known already: Sperm DNA fragmentation is associated with fertilisation and implantation success. It was suggested that routinely applied methods, such as swim up, lead to selection of spermatozoa with low DNA fragmentation. Another technique for sperm selection is based on sperm zona pellucida binding. The sperm zona adhesion abilities were suggested as a predictor for the fertilisation ability of the semen. Recent study has shown that using sperm bound to zona pellucida proteins for ICSI leads to higher pregnancy rates and fewer miscarriages than conventional sperm selection. However, data about sperm DNA fragmentation after zona pellucida selection is still absent.
Study design, size, duration: This observational study involved 29 normozoospermic patients of MHAT "Nadezhda", Sofia, Bulgaria, between December 2019 and January 2020. Semen samples were analysis performed according to WHO 2010. Native semen was liquefied and was subjected to swim up. The isolated motile spermatozoa were allowed to adhere on acid solubilized zona pellucida coated petri dishes. Sperm DNA fragmentation (SDF) was analysed in each patient's native semen, swim up prepared samples and zona bound spermatozoa.
Participants/materials, setting, methods: SDF of fresh semen, swim up prepared samples and zona bound spermatozoa was done using Halosperm G2 test kit (Halotech DNA SL, Madrid, Spain). Data was checked for normal distribution using Kolmogorov-Smirnov test. Spearman correlation analysis was used for comparison of the SDF and semen analysis parameters. Differences between the groups were *analyzed by Wilcoxon* paired test. Statistical analysis was performed using IBM SPSS ver.21. P<0.05 was considered significant.
Main results and the role of chance: The percentage of SDF of fresh semen, swim up prepared samples and zona bound spermatozoa did not correlate to the conventional semen analysis parameters (sperm concentration, motility and morphology) of the studied normozoospermic patients (p>0.05). However, the SDF values of the fresh semen were found to be 32±5%, which is slightly above the previously estimated cut off for good DNA fragmentation (30%).

Following sperm preparation by swim up and zona binding the SDF values were reduced significantly (p=0.018, p=0.017 respectively). Moreover, the median SDF percentage in the swim up prepared samples and zona bound spermatozoa were found to differ significantly (Median: 15% vs. 10%; p=0.01). In addition, zona bound spermatozoa from all patients had DNA damage 3-4 times lower than the estimated cut off value for good SDF.**Limitations, reasons for caution:** Results from this study need to be confirmed by larger group of samples.
Wider implications of the findings: Sperm zona selection provides spermatozoa with good DNA fragmentation. Zona selection technique may be used to improve the impact of abnormal SDF on the reproductive outcomes even in normozoospermic patients.
Trial registration number: not applicable

P-073 Effect of Unilateral Microsurgical Varicocelectomy on fertility outcome and treatment plans of patients with severe oligospermia

H. Elbardisi[1], K. Khalafalla[1], A. Majzoub[1], S. Alsaid[1], M. Arafa[1]

[1]Hamad Medical Corporation, Urology, Doha, Qatar

Study question: Does unilateral microsurgical subinguinal varicocelectomy have a favorable effect on the fertility potential of patients with severe oligospermia?
Summary answer: Microsurgical sub inguinal varicocelectomy improves semen parameters of patients with severe oligospermia improving their fertility outcome.
What is known already: Severe oligospermia or a sperm concentration below 5 million/ml, is an extreme derangement of male fertility potential that is often managed with assisted reproductive therapy. Intracytoplasmic sperm injection (ICSI) is usually preferred in such type of patients in order to maintain a higher success rate during the invitro fertilization (IVF) cycle. The effect of Varicocelectomy on patients with oligoasthenoteratospermia has been extensively investigated with proven improvement, however its impact on patients with severe oligospermia remains to be elucidated.
Study design, size, duration: This was a retrospective study of 114 patients diagnosed with severe oligospermia between January 2016 till January 2018, who underwent unilateral microsurgical sub inguinal varicocelectomy. Patients with genetic abnormalities, history of varicocelectomy, or those who received any infertility related therapy before surgery were excluded from the study.
Participants/materials, setting, methods: Patients were evaluated pre-operatively and 6 months following surgery. Data regarding age, testicular size, serum hormones (Testosterone, LH, FSH) and semen analysis results were collected. Categorical data was presented as numbers (percentages) while numerical data was presented as mean ± SEM. Data was compared using Wilcoxon Signed Ranked Test. A p value <0.05 was considered significant.
Main results and the role of chance: The patients' mean age was 36.8 ± 5.2 years. A statistically significant improvement in sperm concentration (p<0.001), total motility (p<0.001) and progressive motility (p=0.003) was observed after surgery. The pre-surgical total motile sperm count (TMSC) was 1.5±0.34 million sperm and was significantly increased following surgery to 10.5±2.0 million sperm (p< 0.001). No significant differences were noted in hormonal levels following surgery.

Six months following varicocelectomy, the sperm concentration improved in 79 patients (69.3%); 16/114 (14%) patients showed normal sperm concentration while 26/116 (22.8%) patients improved to the oligospermia category. The TMSC increased to above 5 million in 28 patients (24.5%).
Limitations, reasons for caution: The main limitation is the retrospective design of the study.
Wider implications of the findings: Documenting an improvement in the fertility potential of patients with severe oligospermia is important as it would change their management plans. A good percentage of patients may become candidates for intrauterine insemination or even natural conception instead of intracytoplasmic sperm injection.
Trial registration number: NA

P-074 Effect of Abstinence Period on Seminal Oxidative Stress in Infertile men

M. Arafa[1], K. Khalafalla[1], A. Majzoub[1], A. Agarwal[2], S. Alsaid[1], H. Elbardisi[1]

[1]Hamad Medical Corporation, Urology, Doha, Qatar ; [2]Cleveland clinic, American Center for Reproductive Medicine, Cleveland-, U.S.A.

Study question: Does the duration of abstinence period affect seminal oxidative stress in infertile men?
Summary answer: Shorter abstinence period significantly decreases seminal oxidative stress with subsequent improvement of motility, progressive motility and normal morphology.
What is known already: The duration of abstinence may affect the semen quality. While longer abstinence is associated with more semen volume and sperm concentration, it negatively affects total and progressive motility. WHO guideline stated that abstinence should be 2-7 days. Different studies have sought to determine the optimal time frame for ejaculatory abstinence, however the results are often found to be contradictory. Also, there are no studies discussing the effect of abstinence on seminal oxidative stress measured by oxidation reduction potential.

Study design, size, duration: This retrospective study included 255 patients presenting with male factor infertility to a tertiary medical center over a period of 2 months. The inclusion criteria were patients who did semen analysis with an abstinence of less than 2 days or more than 5 days.
Participants/materials, setting, methods: Patients were grouped into group A (76 patients) with abstinence < 2 days and group B (177 patients) with abstinence > 5 days. Semen analysis was done according to the 5th edition WHO guidelines. ORP was determined using the MiOXSYS system (Aytu BioScience, Englewood, CO). SDF was measured by sperm dispersion method (Halosperm). The results were compared by Wilcoxon rank sum test and paired T test. p value < 0.05 was considered significant.
Main results and the role of chance: The patients' mean age was 35.9±7.6 years. ORP was significantly lower in group A than in group B (2.5±3.0 vs 5.3±10.5mV/10^6 sperm). Sperm concentration was lower in group A than group B but the difference was non-significant (32.9±25.7 vs 37.7±28.6millions/ml). Total and progressive motility were also better in group A than group B (53.6±18.1% vs 49.2±18.8%, 12.5±10.9% vs 11.6±11.5% respectively) but again the differences were insignificant. Abnormal form was slightly and insignificantly less in group A than group B (93.7±9.8% vs 93.8±9.0%). Sperm DNA fragmentation was less in group A than group B (28.8±21.2% vs 31±15.9%) but the difference was not significant.
Limitations, reasons for caution: The main limitation is the retrospective design of the study.
Wider implications of the findings: The significant improvement in seminal oxidative stress with the short abstinence may introduce a new treatment technique in infertile men were couples can be counseled to engage in frequent sexual intercourses hoping to benefit from the improvement in semen parameters.
Trial registration number: NA

P-075 Threshold-dependent effects of energy restriction as a novel potential therapy to increase sperm concentration in men with obesity: A Randomised Controlled Study

"Abstract withdrawn by the authors"

P-076 Does the presence of varicocele Influence microsurgical testicular sperm retrieval in men with non-obstructive azoospermia (NOA)?

K. Khalafalla[1], M. Arafa[1], H. Elbardisi[1], S. Alsaid[1], A. Majzoub[1]

[1]Hamad Medical Corporation, Urology, Doha, Qatar

Study question: Can the outcome of sperm retrieval in patients with non-obstructive azoospermia be affected by an untreated clinical varicocele?
Summary answer: The presence of clinical varicocele did not influence the sperm retrieval rate in this population of NOA patients.
What is known already: Varicocele is prevalent in approximately 5% of men with NOA, the pathophysiology of azoospermia and its relation to varicocele is still under investigation. It is still debatable whether varicocele contributes to spermatogenesis disruption and affects sperm retrieval rates in NOA. Recent studies have reported a significant influence for varicocele on patients with non-obstructive azoospermia (NOA) suggesting varicocelectomy prior to testicular sperm extraction (TESE) procedures.
Study design, size, duration: The charts of 448 patients who presented to the Male infertility clinic from 2011 – 2016 with Non-obstructive azoospermia and underwent microsurgical TESE were included and retrospectively studied. Exclusion criteria for this study were prior varicocelectomy, TESE, chemotherapy or radiation therapy, patients with abnormal genetics or chromosomal deletions.
Participants/materials, setting, methods: Patients demographics, clinical and laboratory data (age, BMI, estradiol, FSH, LH, Prolactin, Testosterone) as well as the sperm retrieval outcome were compared between non-obstructive azoospermic patients with and without varicocele. Data was compared using Wilcoxon Signed Ranked Test. A p value <0.05 was considered significant.
Main results and the role of chance: Varicocele data was available in 222 charts, 157 patients had a clinical varicocele and 70 had no varicocele. Sperm were retrieved from 57% of patient with Varicocele (n=89) compared with 45.3% of patients (n=38) with No-Varicocele. The difference in sperm retrieval between both groups was not statistically significant (p=0.09). Patients with varicocele were significantly older than patients with No-varicocele (p=0.006). No significant differences between the 2 groups with regards to testicular size (right, left), reproductive hormones (Testosterone, LH, FSH, Estradiol) and BMI were observed.

Parameters	Non Varicocele group	Varicocele group	P Value
Age (years)	35.3±0.74	38.2±0.623	0.006
BMI (Kg/m²)	31.5±1.6	30.4±0.8	0.12
Testosterone (nmol/L)	14.8±1.06	15.8±0.62	0.38
LH (IU/L)	7.23±0.62	7.03±0.43	0.79
FSH (IU/L)	13.85±1.26	13.53±0.78	0.83
E2 (pg/ml)	110.15±5.8	122.5±7.4	0.296
Left Testes size (cm³)	7.02±0.54	7.06±0.3	0.94
Right Testes size (cm³)	7.9±0.6	7.0±0.3	0.12

Limitations, reasons for caution: The main limitation is the retrospective design of the study.
Wider implications of the findings: Patients with NOA who have a clinical varicocele should be thoroughly counselled before treatment decisions are made. The expected clinical benefit in face of the invasiveness and the extra cost of the varicocelectomy should be discussed with the patients before undergoing testicular sperm retrieval.
Trial registration number: NA

P-077 Impact of the assessment of sperm DNA damage on IVF and ICSI outcome: role of intra-individual variation

P. Christensen[1], E. Parner[2], A. Birck[3]

[1]SPZ Lab A/S, Clinical Research, Copenhagen, Denmark ;
[2]Aarhus University, Department of Public Health- Section for Biostatistics, Aarhus C, Denmark ;
[3]SPZ Lab, Clinical Research, Copenhagen OE, Denmark

Study question: How does intra-individual variation in the assessment of sperm DNA damage affect the outcome of IVF and ICSI treatments?
Summary answer: Intra-individual variation in assessment of sperm DNA damage plays a very important role for the outcome of IVF and ICSI treatments.
What is known already: The impact of sperm DNA damage on the outcome of assisted reproduction remains controversial. The lack of agreement in the literature is partially due to a diversity of test methods as well as poorly standardized protocols. Methods based on flow cytometry have the potential of being very robust and precise. However, large differences in intra-individual variation have been reported for different laboratories assessing sperm DNA damage based on the SCSA protocol. Some of this variation may have a biological background, but can to a large extend be due to differences in laboratory performance and quality control procedures.
Study design, size, duration: Simulated power calculations using 10,000 replications were based on our previous retrospective analysis of the impact of sperm DNA damage on 406 clinical cases for IVF and ICSI (Christensen et al., Hum Reprod. 2013;28:i128-P-026). Intra-individual variation (CV within male: CV_w) for assessment of sperm DNA damage in our laboratory was calculated to 16.5% using data from a previous randomized and double-blind controlled trial (Blomberg-Jensen et al., J Clin Endocrinol Metab 2018;103:870-81).
Participants/materials, setting, methods: The median percentage of sperm with damaged DNA (DFI) was 15.9% with a total CV of 60.2%. Calculations of true pregnancy rates for IVF and ICSI were estimated using a CV_w of 0.0%. The true pregnancy rates were then used in calculations of statistical power. We considered scenarios of CV_w of 16.5% plus increases of CV_w with 50% and 100%. A 2-sided chi2-test was used for comparison of pregnancy rates (significance level 0.05, power 80%).

Main results and the role of chance: For IVF treatments, true pregnancy rates (CV_w = 0) were calculated to 47.7% (DFI≤15) and 22.2% (DFI 15 to 25). For ICSI treatments, true pregnancy rates were calculated to 49.5% (DFI<25) and 24.7% (DFI≥25). Using our laboratory's CV_w of 16.5%, the sample size needed for comparison of pregnancy rates was estimated to n=169 for an IVF study. A study with ICSI-treatments would require a sample size of n=336. When CV_w was increased with 50%, the required sample sizes increased to n=207 (IVF) and n=437 (ICSI). A 100% increase in CV_w gave corresponding sample sizes of n=260 (IVF) and n=569 (ICSI). A small intra-individual variation in the assessment of sperm DNA damage is essential for correct diagnosis and treatment. In contrast, a high variation may lead to misclassification of patients and potentially suboptimal treatment. When the impact of sperm DNA damage on assisted reproduction outcomes is to be studied, a high degree of intra-individual variation increases the required sample size markedly. Without a sufficient sample size, the true biological relationship may remain concealed. To produce reliable and valuable results having low intra-individual variation, laboratories performing tests of sperm DNA damage should ensure good quality control to minimize potential laboratory errors.
Limitations, reasons for caution: None.
Wider implications of the findings: Laboratories performing test of sperm DNA damage should declare the level of uncertainty of the test used. Differences in quality control procedures between laboratories are likely to play a significant role in interpretation of results even when the same method is used and may impact assisted reproduction treatment outcome.
Trial registration number: Not applicable

P-078 Is there an effect of artificial oocyte activation on euploidy of embryo in patients with male factor infertility?

H. Jeong[1], K.Y. Kang[1], H.S. Choi[1], E.H. Moon[1], Y.R. Park[1], S.H. Lee[1], E.A. Park[1], J.Y. Lee[1], H.S. Kim[1], K.A. Lee[2], J.J. Ko[2], I.S. Kang[1], M.K. Koong[1], Y.S. Kim[1], M.J. Kim[1]

[1]CHA Fertility Center Seoul Station, Fertility laboratory, Seoul, Korea- South ; [2]CHA University, Department of Biomedical Science- College of Life Science, Pocheon, Korea- South

Study question: The purpose of this study is to identify euploidy rate in severe male factor patients treated with artificial oocyte activation(AOA) after ICSI.
Summary answer: There was an insignificant difference of euploidy in severe male factor groups treated AOA after ICSI with preimplantation genetic testing-aneuploidy(PGT-A).
What is known already: In many studies, it is known that sperm abnormalities result in abnormal sperm decondensation, aberrant pronuclear development, migration, apposition and first mitosis. It has been reported that treatment of AOA in patients with male factors improves these problems. Also, AOA leads to a cascade of events including extrusion of the second polar body, decondensation of a haploid set of chromosomes and initiation of embryonic development. Thus, sperm abnormalities and oocyte activation are important in fertilization. Generally, AOA is used for low quality oocytes and sperm, and the low fertilization rate and frequent fertilization failure.
Study design, size, duration: 25,427 IVF-ICSI cycles were performed at our center from January 2016 to December 2019. From those studies, only 2,285 cycles were performed with preimplantation genentic testing and 750 cycles were performed with PGT-A. Among them, the cycles with artificial activation after ICSI were only 103 cases. The embryo euploidy rate was evaluated in cases of blastocyst PGT-A. Statistical analysis was performed using the chi-squared test. P values p<0.05 were considered significant.
Participants/materials, setting, methods: IVF-ICSI cycles that performed blastocyst biopsies were sorted from cycles performed with PGT-A and these were divided into four groups depending on the concentration of the sperm; Group 1: severe oligozoospermia(<1xml), Group 2: oligozoospermia(1≤~<15xml), Group 3: normal(≥15xml), Group 4: TESE sperm(sperm retrieved from azoospermia patients). Each group was additionally divided into two groups; with or without activation. We used 10μM/L calcium ionophore(A23187; Sigma-Aldrich) in culture medium for the AOA for 5min at 30min after ICSI.
Main results and the role of chance: Euploidy rates of PGT-A blastocyst with AOA and without AOA after ICSI were compared in each group. Maternal and paternal age had no significant differences between two groups, with AOA and without AOA. Euploidy rates of each group are below. (Groups with AOA- Group 1: 34.88%(43/15), Group 2: 33.33%(78/26), Group 3: 32.46%(228/74), Group 4: 38.71%(31/12), Groups without AOA- Group 1: 30.77%(13/4), Group 2: 33.33%(48/16), Group 3: 34.36%(2363/812), Group 4: 30.77%(26/8)). In severe male factor group, Group 1 and Group 4, cases with AOA had higher euploidy rate than cases without AOA. However, there was no significant difference stastistically in Group 1(34.88% vs. 30.77%, *p>0.05*) and Group 4(38.71% vs. 30.77%, *p>0.05*). In oligozoospermia group, Group 2, euploidy rate of cases with AOA was no different from cases without AOA(33.33% vs. 33.33%, *p>0.05*). Also, there was no significant difference in normal sperm group, Group 3(32.46% vs. 34.36%, *p>0.05*). This study showed no significant differences in euploidy rate between cases with and without AOA.
Limitations, reasons for caution: Even though there were numerous cases in our center, we had limits in analyzing significant differences among cases. It is because there were a few PGT-A cases with AOA in male factor infertility patients. Furthermore, we only concerned cases treated with calcium ionophore among various AOA in this study.
Wider implications of the findings: Although our study needs to be supported by further study, this study revealed that AOA contributes to increasing euploidy rates in severe male factor infertility patients. Consequently, it is expected that AOA could increase clinical pregnancy rates by increasing euploidy rates in severe male factor infertility patients.
Trial registration number: not applicable

P-079 Very low concentration of aged cerium dioxide nanoparticles (CeO2NPs) induce DNA damage in human and rat gametes after in vitro exposure

M. Cotena[1], M. Auffan[2], V. Tassistro[1], C. Uboldi[1], J. Rose[2], J. Perrin[3]

[1]Faculty of Medecine- Aix Marseille Université- Equipe Biomarqueurs-Environnement- Santé - Institut Méditerranéen de Biodiversité et d'Ecologie UMR 7263- IRD 237, Faculty of Medicine, Marseille, France ; [2]European Centre Research And Teaching In Geosciences of the Environment CEREGE - CNRs- IRD- Collège de France- INRA- Aix-en-Provence- France, ED-Environnement Durable, Marseille, France ; [3]Aix Marseille University- AP-HM La Conception- Centre Clinico-Biologique d'Assistance Médicale à la Procréation - CECOS- 13385 Marseille Cedex 5- France, Faculty of Medicine, Marseille, France

Study question: Do aged CeO_2NPs induce DNA damage in human and rat gametes after *in vitro* exposure?
Summary answer: *In vitro* gametes exposure to the lowest concentration of CeO_2NPs tested induces a significant increase in DNA damage compared to the highest doses.
What is known already: Cerium dioxide nanoparticles (CeO_2NPs) are an active catalyst and are widely used as diesel additive to increase fuel economy. They are released in the atmosphere after engine combustion. The Organization for Economic Cooperation and Development included CeO_2NPs in the priority list of nanomaterials requiring urgent evaluation. CeO_2NPs can transfer to the testicles and epididymis after inhalation in rats. *In vitro* exposure of human and mouse sperm and cumulus oocyte complexes (COC) to low concentrations of pristine CeO_2NPs (10 $\mu g.l^{-1}$) induce significant DNA damage. Nevertheless, pristine CeO_2NPs are altered by combustion and the potential hazard of aged CeO_2NPs exposure remains unexplored.
Study design, size, duration: Pristine CeO_2NPs were extracted from Envirox™ diesel additive, combusted at 850°C, (average combustion temperature in a diesel engine) and physically characterized. Chemical (un)stability in Ferticult™ embryo culture medium was assessed by Inductively coupled plasma mass spectrometry (ICP-MS). Rat gametes were collected in epididymis and oviducts after cervical dislocation euthanasia of 60 days old males and 26 days old females (after ovarian stimulation). Human frozen sperm from fertile donors were purchased from Germetheque biobank (France).
Participants/materials, setting, methods: Human and rat gametes were exposed *in vitro* to CeO_2NPs [1 to 1.10^3 $\mu g.l^{-1}$] during 1 hour at 37°C, 5%CO_2. DNA damage was analysed by alkaline comet assay (ACA) and quantified by Olive Tail Moment (OTM) in COC and by %Tail DNA in sperm. CeO_2NPs-cells

interaction was assessed in human sperm by Transmission Electron Microscopy (TEM). Oxidative stress was evaluated using 2,7-dichlorodihydrofluorescein (H_2DCF) probe detected by Flow Cytometry and expressed as Mean Fluorescence Intensity (MFI).

Main results and the role of chance: In human sperm, exposure to 1 µg.l^{-1} CeO_2NPs induced a significant increase of DNA damage (mean %Tail DNA±SEM = 32.06 ± 0.54) compared to the unexposed control (11.96 ± 0.30) ($p < 0.0001$). TEM analysis showed big CeO_2NPs aggregates at 100 µg.l^{-1} but close proximity between cells and NPs at 1 µg.l^{-1}. Oxidative production was increased in human sperm exposed to 1 µg.l^{-1} CeO_2NPs (MFI±SEM = 16.9% ± 1.5), compared to the negative control (2.7% ± 1.4).

In rat oocytes and follicle cells, exposure to 1 µg.l^{-1} CeO_2NPs induced significantly higher DNA damage (mean±SEM OTM = 10.26±0.36 and 4.91±0.29, respectively) compared to the unexposed controls (2.21±0.18 and 0.72±0.05, respectively) and to higher CeO_2NPs concentrations ($p < 0.0001$). In rat sperm, exposure to 1 µg.l^{-1} CeO_2NPs induced significantly higher DNA damage (mean %Tail DNA±SEM = 21.23±0.37) compared to unexposed control (11.06±0.24) and to higher CeO_2NPs concentrations ($p < 0.0001$).

DNA damage was inversely proportional to the CeO_2NPs concentration; we might hypothesize that our results are related to the aggregation states. At high concentration, nanoparticles tend to aggregate between them, reducing the surface of interaction with cells. Conversely, at low concentration they can easily come into contact with cells.

Limitations, reasons for caution: These results cannot be extrapolated to *in vivo* toxicity of CeO_2NPs after inhalation, but demonstrate that interactions between CeO_2NPs and germ cells induce significant DNA damage and oxidative stress. Additional data should be needed to better understand the mechanism of interaction between cells and NPs by using nano-tomography imaging analysis.

Wider implications of the findings: The results obtained give some light on the complex cellular mechanisms by which ROS generation could exert their biological effects on human spermatozoa after CeO_2NPs *in vitro* exposure. Potential impacts of diesel exhaust exposure in couples are a major concern for public health, highlighting the need for *in vivo* studies.

Trial registration number: 15447-20180611102119 50

P-080 Air pollution and seminal parameters in subfertile men

"Abstract withdrawn by the authors"

P-081 Correlation between teratozoospermia index (TZI) and sperm aneuploidy in infertile men

M. Benincasa[1], A. Crippa[1], M.C. Magli[1], F. Coppola[2], G.B. La Sala[1], L. Gianaroli[1]

[1]*S.I.S.Me.R, Reproductive Medicine, Bologna, Italy ;*
[2]*Centro Medico Spallanzani, Clinical Center, Parma, Italy*

Study question: Does the teratozoospermia index (TZI) correlate with the frequency of sperm aneuploidy in a manner that is dependent on the severity of the male factor condition?

Summary answer: TZI correlates with the frequency of sperm aneuploidy and increases with the severity of the male factor condition.

What is known already: The contribution of sperm aneuploidy to the embryo chromosome status is generally perceived to be clinically irrelevant in comparison with the oocyte counterpart. However, increases in sperm aneuploidy have been reported to have an effect on male infertility and on IVF cycle outcomes especially in cases of repeated implantation failure and recurrent miscarriages. Infertile men typically have significantly higher levels of sperm aneuploidy compared with fertile or normozoospermic males and the level of aneuploidy correlates to the severity of the male factor. Sperm aneuploidy is routinely evaluated by FISH (Fluorescence in Situ Hybridization).

Study design, size, duration: Since January 2018, 139 sperm samples from patients with a normal karyotype undergoing assisted conception cycles were analysed for semen parameters according to WHO 2010. In addition to the morphology evaluation, TZI (representing the number of abnormalities per abnormal spermatozoon) was also determined. The same sperm samples were also analyzed by FISH to evaluate possible correlations between TZI, aneuploidy and the severity of the male factor.

Participants/materials, setting, methods: All patients had a history of reproductive implantation failures and/or miscarriages. When classified according to WHO criteria, 29 samples were normozoospermic, 82 were moderate oligoasthenoteratozoospermic (OAT) (sperm count ≥5 million and < 15 million spermatozoa/ml) and 28 were severe OAT (sperm count <5 million spermatozoa/ml). The FISH analysis was performed for nine chromosomes using probes specific for the chromosomes X, Y, 13, 15, 16, 17, 18, 21 and 22.

Main results and the role of chance: TZI was significantly higher in severe OAT samples (2.1±0.2, range 1.5-2.4) when compare with normal (1.7±0.1, range 1.4-1.9) (P= 1.11E-8) and moderate OAT samples (1.9±0.2 range 1.5-2.5) (P= 2.20E-5). TZI was also significantly higher in moderate OAT compared with normal samples (P= 1.19E-6).

The frequency of total sperm aneuploidy was in inverse correlation with sperm concentration (R= -0.38, P= 5.36E-6), progressive motility (R= -0.41, P= 4.57E-7), total motility (R= -0.25, P= 0.0026) and morphology (R= -0.36, P= 1.04E-5. When considering the sperm typology, the frequency of total aneuploidy was significantly higher in severe OAT (5.01±1.82%, range 2.62-9.04%) when compared with normal (2.24±0.79%, range 0.63-4.22%) (P= 9.31E-9) and moderate OAT samples (3.04±1.12%, range 1.47-7.82%) (P= 2.50E-6). It was also significantly higher in moderate when compared with normal samples (P=0.00067). The combined analysis of TZI and aneuploidy in the different sperm typologies showed a significant positive correlation between the frequency of total aneuploidy and TZI (R= 0.47, P= 6.72E-9).

Limitations, reasons for caution: This study included a restricted number of samples. Additional data are necessary to corroborate our findings.

Wider implications of the findings: Morphologically abnormal spermatozoa often have multiple defects. A detailed assessment of their incidence would refine the relevance of morphological defects, single or multiple, on the capacity of a sperm cell to support fertilization and embryo development, contributing to the evaluation of the extent of damage occurring during human spermatogenesis.

Trial registration number: Not Applicable

P-082 Identification of novel mutations in DPY19L2 responsible for human fertilization failure and have a favorable clinical outcomes by artificial oocyte activation

X.J. Zhuang[1], P. Liu[1]

[1]*Reproductive Medical Center- Peking University Third Hospital, Department of Obstetrics and Gynecology, Beijing, China*

Study question: Does artificial oocyte activation (AOA) together with intracytoplasmic sperm injection (ICSI) improve clinical outcomes for human fertilization failure of globozoospermia linked to DPY19L2 mutations patients?

Summary answer: Novel DPY19L2 deletions were revealed by whole-exome sequencing. AOA together with ICSI improved cycles of DPY19L2 mutations by increasing the fertilization and transferable embryo rates.

What is known already: In clinical, some infertile couples suffer from recurrent fertilization failure. Globozoospermia is a rare form of male infertility characterised by round-headed sperm and malformation of the acrosome. Although the fertilization rate was improved by ICSI, about 1–5% of ICSI cycles still display fertilization failure. The genetic reasons for fertilization failure are largely unknown. Although pathogenic variants in DPY19L2 are known causes of globozoospermia, novel DPY19L2-mutated in infertile patients should be revealed and the treatment of these DPY19L2 deleted globozoospermic patients by AOA together with ICSI should be evaluate.

Study design, size, duration: Globozoospermic patients were recruited from the Reproductive Medical Center of Peking University Third Hospital (Beijing China) between January 2016 and June 2019. Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committees of Peking University Third Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Participants/materials, setting, methods: DPY19L2 were analyzed with blood and sperm samples from globozoospermic patients. DPY19L2 levels in human testes were investigated using RT-PCR, western blotting and Immunofluorescent analysis, respectively. And its novel mutations using

whole-exome sequencing and sanger sequencing was explored. Human sperm samples from globozoospermic donors with DPY19L2 mutations are subjected to ICSI and AOA. The fertilization rates, cleavage rates and transferable embryo rates of DPY19L2-mutated patients were compared with its previous ART cycles.

Main results and the role of chance: DPY19L2 mRNA and protein were abundantly transcribed in male testis in human. Immunohistochemical results revealed DPY19L2 was localized in the cytoplasm of spermatids and round spermatids. Through whole-exome sequencing and sanger sequencing, we identified that five patients carried DPY19L2 deletions and six patients contained novel DPY19L2 point mutations in couples diagnosed with fertilization failure. Expression of these mutations in human testis significantly reduced the levels of DPY19L2 expression. In addition, the fertilization rate, embryo cleavage rate and transferable embryo rate were significantly higher in the AOA group than its previous cycle of group (fertilization rate 50.38% versus 13.86%, respectively, $P < 0.001$; embryo cleavage rate 59.16% versus 19.04%, respectively, $P < 0.001$; transferable embryo rate 43.51% versus 16.69%, respectively, $P < 0.001$). Five live-birth babies were born by AOA together with ICSI.

Limitations, reasons for caution: Analysis was performed in testicular biopsy samples from only a small number of patients with DPY19L2 mutations. In future investigations, a larger sample size should be used and the role of the other genes involved in the globozoospermic should be analyzed.

Wider implications of the findings: DPY19L2 variants are highly related to globozoospermia and fertilization failure. AOA could rescue the phenotype of fertilization failure and help establish pregnancy and lead to live birth. These findings bring us closer to a complete molecular diagnosis for globozoospermia patients which would help to predict the success of reproductive treatments.

Trial registration number: This study was supported by the National Natural Science Foundation of China (NO.81671513) and Beijing Natural Science Foundation (NO.7172236).

P-083 Does sperm origin - either ejaculated or testicular – affect early and late embryonic morphkinetic parameters

G. Karavani[1], N. Schachter - Safrai[1], I. Har-Vardi[2], A. Buxboim[3], Y. Kantor[4], S. Zeev[4], O. Yuval[4], S. Yoel[5], A. Ben-Meir[1]

[1]Hebrew University-Hadassah Medical center, Obstetrics and Gynecologyresident, jerusalem, Israel ;
[2]Soroka, The IVF Unit Gyn/Obs- Soroka University Medical Center- Israel- Faculty of Health Sciences- Ben-Gurion University of the Negev- Beer-Sheva- Israel., Beer Sheva, Israel ;
[3]Hebrew University-Hadassah Medical center, Alexander Grass Center for Bioengineering- School of Computer Science and Engineering- The Hebrew University of Jerusalem- Jerusalem- Israel, jerusalem, Israel ;
[4]The Hebrew University of Jerusalem- Jerusalem- Israel., Alexander Grass Center for Bioengineering- School of Computer Science and Engineering, Jerusalem, Israel ;
[5]Infertility and IVF Unit, Helen Schneider Hospital for Women, Rabin Medical Center

Study question: Is there an effect of sperm origin on embryo morphokinetics in couples diagnosed with male factor infertility (MFI)?

Summary answer: Testicular sperm impacted early developmental stages, while ejaculated sperm was associated with faster late cell division, faster compaction and higher implantation rate.

What is known already: Time-lapse imaging provides a non-invasive technique that enables assessment of embryo quality by morphokinetic parameters. While embryonic morphokinetic milestones are known to reflect the developmental and implantation potential, it is unclear whether sperm origin, either ejaculated or testicular, in MFI has an early or late effect with influence on cleavage, blastulation and implantation potential. There is only scarce data regarding the paternal effect on embryo development, with regard to sperm origin, using TLI.

Study design, size, duration: The study included a retrospective analysis of morphokinetic parameters performed by TLI from five medical centers between January 2013 and December 2017.The developmental process and kinetics of 424 embryos obtained from ejaculated sperm from couples with MFI attributed to Oligoasthenoteratozoospermia (OAT) were compared to 160 embryos derived from surgically extracted testicular sperm from couples diagnosed with azoospermia (AS)

Participants/materials, setting, methods: The study included women younger than 38 years who underwent controlled ovarian stimulation and fertilization using either ejaculated abnormal sperm (OAT) or surgically extracted testicular sperm (AS). A comparison between the morphokinetic parameters, implantation and clinical pregnancy rates of the two groups was made with additional subgroup analysis according to the implantation status. Logistic regression was conducted to assess the association between sperm origin and embryonic morphokinetic parameters and implantation.

Main results and the role of chance: Implantation rate was significantly higher in the ejaculated sperm group compared to the testicular sperm group (45.8% vs. 33.6%, p=0.02). Embryos from the OAT group reached the later morphokinetic milestones - embryonal third cell cycle (ECC3), synchronous division (S3) and Morula compaction- faster than embryos obtained from azoospermic patients. Implanted embryos developed in similar rate in both group. In a multivariate analysis for the entire study population, ejaculated sperm (OR=5.88 (CI 95% 2.56-12.50), p<0.001) and time to 8 cell stage (OR=0.90 (CI 95% 0.82-0.98), p=0.013) were positively associated with successful implantation.

Limitations, reasons for caution: The two patients' groups were not homogeneous in their basic characteristics. A higher rate of nulliparity and increased fragmentation was demonstrated in the AS couples. Information regarding the maximal dose of GT obtained, previous IVF response and ovarian reserve testing was lacking.

Wider implications of the findings: Ejaculated sperm impact on late preimplantation development, especially the third cell cycle and compaction, may highlight another pathophysiology by which sperm origin effects embryo developmental kinetics. Morphokinetic parameters, with regard to sperm source, may assist in predicting implantation.

Trial registration number: Kamin fund

P-084 Sperm fatty acid profile in infertile men.

A. Stendardi[1], S.E. Pizzasegale[1], F.L. Vellucci[1], G. Collodel[2], E. Moretti[2], C. Signorini[2], L. Gambera[1]

[1]A.G.I. Medica, Reproductive Medicine, Siena, Italy ;
[2]Università degli Studi di Siena, Dipartimento di Medicina molecolare e dello sviluppo, Siena, Italy

Study question: Are the sperm characteristics correlated with sperm fatty acid composition?

Summary answer: Our results show a relationship between sperm fatty acid composition and sperm characteristics evaluated by light and electron microscopy

What is known already: In sperm membrane and semen plasma, lipids and fatty acid (FA) composition are relevant to spermatozoa function and spermatogenesis. Mammalian spermatozoa are characterized by a high proportion of polyunsaturated fatty acids (PUFA) which play a crucial role in sperm maturation and motility, in acrosome reaction and fertilization. Due to the high presence of PUFA, sperm are susceptible to lipid peroxidation. It is known that men with altered seminal parameters had shown different FA composition and different omega-6/omega-3 PUFA ratio.

Study design, size, duration: The proposed investigation is a retrospective study. Twenty-three infertile men (aged 27-38) referred to *AGI Medica* lab (Siena, Italy) for semen analysis were enrolled from January 2019 to June 2019.

Participants/materials, setting, methods: Semen characteristics were evaluated by light and transmission electron microscopy (TEM). TEM data was quantified with a mathematical formula which provides numerical scores as the percentage of apoptosis, necrosis and immaturity and a fertility index. Sperm FA composition was evaluated by a gas chromatography instrumentation (Lipinutragen, Bologna, Italy). The content of FA and the value of the ratios between different FA were correlated with sperm characteristics (Spearman's Rank Correlation Coefficient).

Main results and the role of chance: Semen analysis showed an impaired semen quality. By TEM investigation, sperm pathologies were increased and fertility index reduced as compared to normal values. A positive correlation was detected between docosahexaenoic acid (DHA), an omega-3 PUFA, and sperm concentration, vitality and fertility index. Necrosis was positively correlated with oleic, vaccenic, palmitoleic acid content, and negatively with DHA and saturated fatty acid/monounsaturated fatty acid ratio. The omega-6/omega-3 ratio negatively correlated with sperm concentration, motility and morphology and

positively with sperm necrosis. Sperm immaturity positively correlated with eicosatrienic acid.

With a view to the role of FA in sperm characteristics, these preliminary data show that, i) DHA content appears strictly related to sperm quality, in particular to sperm vitality; ii) the omega6/omega3 ratio increases in presence of necrosis, as well as eicosapentaenoic acid/arachidonic acid ratio.

These results may be implemented by further studies on the relationship between FA composition and sperm characteristics since a dietary FA supplementation should improve semen quality.

Limitations, reasons for caution: The study group is small and selected groups of patients with different reproductive pathological conditions will to be compared

Wider implications of the findings: A deeper knowledge on sperm FA profile and its correlation with sperm pathology indicate the potential role of personalized nutraceutical treatments to modulate sperm FA composition improving male reproductive efficiency.

Trial registration number: none

P-085 A novel homozygous CEP112 mutation in humans causes male infertility with azoospermia

X. Zhang[1], G. Huang[2], G. Zhang[2], W. Xu[2]

[1]Department of Obstetrics/Gynecology- Joint Laboratory of Reproductive Medicine SCU-CUHK- Key Laboratory of Obstetric- Gynecologic and Pediatric Diseases and Birth Defects of Ministry of Education- West China Second University Hospital-Sichuan Universi ;

[2]Department of Obstetrics/Gynecology- Joint Laboratory of Reproductive Medicine SCU-CUHK- Key Laboratory of Obstetric- Gynecologic and Pediatric Diseases and Birth Defects of Ministry of Education- West China Second University Hospital-Sichuan Universi

Study question: To date, several gene mutations have been identified in azoospermia, which can explain the genetic causes of a small number of azoospermia cases. Here, we report anovel gene mutations

Summary answer: Our experimental observations on human subjects suggested that CEP112 is involved in sperm flagellum structure and that loss-of-function mutations could lead to male infertility

What is known already: Nearly half of male infertility cases are thought to be linked to genetic defects. And 20% of infertile men are diagnosed with azoospermia. Nonobstructive azoospermia is defined as spermatogenic failure, and are often be discovered during the semen analysis showing the absence of sperm in semen. Azoospermia is a heterogeneous disease with many histological phenotypes. To date, several gene mutations, including TEX11, SYCP3, have been identified in azoospermia, which can explain the genetic causes of a small number of azoospermia cases.

Study design, size, duration: Whole-exome sequencing (WES) was performed with patient DNA. In Brief, genomic DNA was isolated from peripheral blood samples, and utilized for exon capture using the Agilent SureSelect Human All Exon V6 Kit and sequenced on the Illumina HiSeq X system. Candidate pathogenic variant on the patient was validated by Sanger sequencing in the patients' parents as well as the normal controls. Then Knockout mice were used to observe phenotypes.

Participants/materials, setting, methods: A-30-year-old Han Chinese man from a consanguineous family was diagnosed with infertility for 2 years, and no fertility-related diseases were detected in his wife. The parents of the patient were also recruited. Whole-exome sequencing (WES) was performed with patient DNA. Immunofluorescence staining, Scanning electron microscopy (SEM) assay and transmission electron microscopy (TEM) assay were performed according to a protocol described previously. The flow cytometric analysis and cell sorting were performed as previously described.

Main results and the role of chance: To illuminate the genetic cause of the azoospermia in this study, we performed Whole-exome sequencing (WES) was performed on the patient and a homozygous mutation (g.17:64125864_64125941cnv, c.NA, p.NA) was identified in centrosomal protein 112kDa gene named CEP112, which is primarily expressed in the testis and belongs to the cell division control protein 42 effector protein family. The protein encoded by this gene has putative coiled-coil domains and plays a role in spermatogenesis. Our experimental observations on human subjects and mice suggested that CEP112 is involved in sperm flagellum structure and assembly. Loss-of- function mutations could lead to male infertility in humans and mice by damaging spermatogenesis and making abnormal sperm

Limitations, reasons for caution: Considering the relationship between CEP112 and primary ciliarym, we wanted to performed CT and MRI scans on the patient that can assess clinical signs of PCD. Unfortunately, the patient refused further examination, therefore we did not assess ciliary beat in the patient.

Wider implications of the findings: The screening of the deleterious mutations of *CEP112* could be important for clinical molecular diagnosis of male infertility. Further study is required to elucidate the molecular mechanism of *CFAP65* in sperm flagellar development.

Trial registration number: 81771642

P-086 Microfluidic sperm sorter versus sperm slow prior to ICSI in oligoasthenozoospermia samples with poor DNA Fragmentation Index (DFI) scores in SCSA testing

P. Selvaraj[1], K. Selvaraj[1], V. Srinivasan[2], M. Sivakumar[1], V. Harikrishnan[1], S. Balakrishnan[1], S. Sudan[1]

[1]Fertility Research and Women's Speciality Hospital, G.G. Hospital, Chennai, India ; [2]The Tamilnadu Dr.MGR Medical University, Biostatistics, Chennai, India

Study question: Compare the outcomes of sperm preparation by Microfluidics and Sperm slow in oligoasthenozoospermia samples with poor DFI and high DNA stainability scores in sperm chromatin structural assay(SCSA) prior to ICSI.

Summary answer: The use of Microfluidics and Sperm slow had no statistically significant differences on primary outcomes.

What is known already: Ideal sperm preparation methods for oligoasthenozoospermia samples have long been debated especially if additional testing such as DFI and high DNA stainability (HDS) scores have been added using SCSA. Micorfluidics has emerged as an atraumatic method of sperm selection, enabling enhanced retrieval of motile sperm with normal morphology, mimicking nature and proving a non static restricted environment. Spermslow on the other hand has the advantage of replacing Polyvinylpyrrolidone and providing hyaluronan and important components for pre-fertilization event of zona-binding and penetration. This hyaluronan enriched semi viscous medium enhances selection of low DNA fragmented sperm with better embryo formation rates.

Study design, size, duration: A prospective study was conducted from June 2016 to December 2018 with the sample size of 109 couples undergoing assisted reproduction. The mean age of women was 32.26±4.26 years. The mean age of men was 36.62±4.5 years. The oligoasthenozoospermic sample had a mean count of 48.71±13.45 million/ml with motility of 35.13±16.86. The definition for poor SCSA score was a DFI of >50% and HDS of >25 %. The outcomes were interpreted .

Participants/materials, setting, methods: The study participants were divided into two groups. Group A (n=56) comprised of sample prepared by Microfludics and Group B comprised of sample prepared by Sperm slow (n=53). The stimulation protocols were short protocol with antagonist and recombinant hCG for trigger. All patients underwent ICSI. The outcomes in both groups were compared as mentioned for Clinical pregnancy, miscarriage rates and live birth rates.

Main results and the role of chance: There were no statistically significant difference between Group A and Group B in patients who have low DFI value in SCSA test with respect to clinical pregnancy (p=0.112) and miscarriage rates (p=0.61), live birth (p=0.611), fertilization rates (p=0.185), and implantation rates (p=0.623). This signifies that the MFSS and Spermslow gives better normal embryos fertilization and delivery rates equal as per primary study outcomes.

Limitations, reasons for caution: A larger sample size would add more value to the study.

Wider implications of the findings: MFSS and SS have proven clinical efficacy in oligoasthenozoospermic males with poor SCSA scoring. We have achieved better clinical outcomes when compared to our retrospective results using conventional sperm preparation methods in similar groups.

Trial registration number: NA

P-087 Comparison between the sperm retrieval rate by micro-dissection testicular sperm extraction (mTESE) versus convention TESE (mTESE) combined with stereoscopic dissection

A. AlAhwany[1], T. Alglely[2], M. Ragab[1], H. AlAhwany[3]

[1]Cairo University, Department of Andrology, Cairo, Egypt ;

[2]Nile IVF Center, Department of Embryology, Cairo, Egypt ;
[3]Nile IVF Center, Department of Gynaecology, Cairo, Egypt

Study question: Can conventional TESE (cTESE) combined with stereoscopic dissection achieve a sperm retrieval rate similar to Micro-dissection Testicular Sperm Extraction (mTESE)?
Summary answer: Our study suggests that micro-TESE still has the highest recovery rate of sufficient sperms and good quality diatoms for immediate or potential cryopreservation for ICSI.
What is known already: Infertile patients with non-obstructive azoospermia are treated by testicular sperm harvesting combined with ICSI. Micro-dissection Testicular Sperm Extraction (mTESE) is considered the gold standard procedure with the highest sperm retrieval rate compared with the conventional TESE. But mTESE cannot be widely offered in most infertility centres because of its high cost, should be done by an expert well-trained surgeon and requires expensive equipment. Laboratory stereoscopic testicular tissue dissection helps in identifying the dilated seminiferous tubules. In this study, we tested whether cTESE combined by stereoscopic dissection can identify dilated seminiferous tubule and subsequent high sperm retrieval rate.
Study design, size, duration: This is a prospective comparative study, comparing the difference in sperm retrieval rate between Micro-dissection Testicular Sperm Extraction (mTESE) (n=100) and Convention TESE (cTESE) using Stereomicroscope (n=100), where Two hundred patients with NOA undergoing ICSI and TESE were recruited between 2018 and 2019.
Participants/materials, setting, methods: Two hundred patients with NOA undergoing ICSI and TESE were recruited. Patients were counselled, consented and randomly randomised. Convention TESE with additional stereomicroscope dissection was applied to the first group (n=100), while micro-TESE was applied to the second group (n=100). Parameters such as patient's average age, testicle size, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone levels, were analysed as predictors of sperm recovery rate.
Main results and the role of chance: The average age of 100 men in the micro TESE was 35.74 ± 37.7, the size of the right testicle was 10.5 ± 4.5, the size of the left testicle was 10.5 ± 4.3, the level of the hormone FSH recorded 19.3 ± 13.9, and the hormone LH concentration was 17.1 ± 23.8, and the testosterone hormone level was 3.4 ± 2.3. In conventional TESE group the average age of 100 men 35.17 ± 7.83 and the size of the right testicle 10.9 ± 4.6 and the size of the left testicle 11.1 ± 4.9 and the level of the hormone FSH 18.3 ± 10.4 and the hormone LH concentration 21.4 ± 44.1 and testosterone level 2.7 ± 0.7. Micro-TESE was successful in obtaining sperm from the testicle in 43% (43/100) of cases. While patients with sperm retrieval in a conventional TESE manner the success rate was 36% (36/100) showing a statistically significant difference (P<0.05).
Limitations, reasons for caution: 1) Larger (n) number of patients were needed. 2) Some of the patients already undergone a partial or conventional TESE screening elsewhere. 3) This study was single-blinded and subjective to the surgeon decision during the operation.
Wider implications of the findings: Even though mTESE shows a higher recovery rate of sufficient sperms for immediate or potential cryopreservation for ICSI, still, conventional TESE combined with stereoscopic dissection could be the method of choice of clinics because of its low cost and also giviving better results than partial or conventional TESE screening.
Trial registration number: N/A

P-088 Does oral antioxidant supplementation for the male partner improve clinical pregnancy rate in couples undergoing ICSI treatment for male factor infertility? A randomized controlled trial

T. Joseph[1], M. Mascarenhas[2], R. Karuppusami[3], M. Karthikeyan[4], A.T. Kunjummen[4], M.S. Kamath[4]

[1]Christian Medical College, Reproductive Medicine, Vellore, India ;
[2]Leeds Fertility -Leeds Teaching Hospitals NHS Trust-, Leeds Fertility-, Leeds-, United Kingdom ;
[3]Christian Medical College and Hospital, Biostatistics, Vellore, India ;
[4]Christian Medical College and Hospital, Reproductive Medicine, Vellore, India

Study question: Does oral antioxidant supplementation for the male partner improve clinical pregnancy rate in couples undergoing intracytoplasmic sperm injection (ICSI) for male factor infertility?
Summary answer: Oral antioxidant supplementation for the male partner resulted in significantly increased clinical pregnancy rate in couples undergoing ICSI for male factor infertility versus no supplementation
What is known already: Reactive oxygen species (ROS)-mediated damage to sperm contributes significantly to male factor infertility. ROS related injury reduces fertilization potential and adversely affects sperm DNA integrity. Antioxidants act as free radical scavengers to protect spermatozoa against ROS induced damage. During ICSI, use of sperms which have been exposed to ROS mediated damage may affect the treatment outcome. Pre-treatment with anti-oxidants may reduce ROS mediated sperm DNA damage. Currently, there is ambiguity regarding role of anti-oxidants before ICSI in male factor infertility due to conflicting results from earlier studies.
Study design, size, duration: This was an open label randomized trial conducted at a tertiary level infertility clinic between Feb 2013-Jan 2019. The trial included 200 subfertile couples who were undergoing ICSI treatment for male factor infertility
Participants/materials, setting, methods: Couples were randomized into treatment arm (n = 100) and control arm (n = 100). In the treatment arm, the male partner received oral anti-oxidants (vitamin C, vitamin E and zinc) for three months just prior to ICSI cycle. In the control arm, no antioxidant was given to the male partner. The primary outcome was clinical pregnancy rate and live birth and miscarriage rates were the secondary outcomes
Main results and the role of chance: A total of 200 randomized women were available for analysis**.** The clinical pregnancy per transfer was significantly higher following anti-oxidant therapy (54.7% vs.36.2% Odds ratio, OR: 2.1, 95% Confidence Interval, CI 1.06-4.26) compared to no therapy. There was no significant difference in live birth rate per transfer (39.1%, vs. 30.4 %; OR: 1.5, 95% CI 0.71-3.00) or miscarriage rate per pregnancy (25.7% vs. 8.0%; OR: 3.9, 95% CI 0.78-20.3) following antioxidant therapy compared to no therapy. However, the intention to treat analysis did not show any significant difference in live birth rate per woman randomized (25.0% vs.21.0%; OR: 1.3, CI: 0.65-2.43). The semen parameters of sperm concentration (18.2 (8.7,37.5) vs. 20.5 (8.1,52.5) million; *P*= 0.86) , motility (34 (20,45) vs. 32.0 (18,45) %; *P* = 0.55) and morphology (2.0 (1.39) vs. 2.2 (1.48); *P* = 0.63) did not show any significant improvement before and after anti-oxidant therapy.
Limitations, reasons for caution: Objective assessment of sperm DNA damage was not done before and after the anti-oxidant supplementation. The study duration got prolonged from two to five years due to slow recruitment. However, no major protocol changes were introduced during the study period
Wider implications of the findings: The preliminary result of the current study suggests that while there is an increase in clinical pregnancy rate following anti-oxidant supplementation for the male partner in couples undergoing ICSI for male factor infertility compared to no supplementation, there is no improvement in live birth rate
Trial registration number: CTRI/2013/02/003431

P-089 Effort to ameliorate sperm profile by folic acid-antioxidant combination therapy to evade single nucleotide polymorphisms of methylenetetrahydrofolate reductase and methionine synthase in idiopathic male infertility

R. Chattopadhyay[1], S. Parvin[2], S. Kalapahar[2], P. Paladhi[3], S. Dutta[3], S. Ghosh[3], P. Chakraborty[2], B. Chakraborty[2]

[1]Institute of Reproductive Medicine, ART, Calcutta, India ;
[2]Institute of Reproductive Medicine, Department of Assisted Reproduction, Calcutta, India ;
[3]University of Calcutta, Department of Zoology, Kolkata, India

Study question: Does anti-oxidants and folate combination therapy influence the association of single-nucleotide-polymorphisms (SNPs) in methylenetetrahydrofolate reductase (MTHFR) and/or methionine synthase (MS) in hyperhomocysteinemic idiopathic male infertility (IMI)?
Summary answer: MTHFR polymorphism with (1298A>C) SNP is associated with hyperhomocysteinemic IMI. Folate-antioxidant/s combination therapy confers no significant improvement in sperm parameter/s in treated cohort.

What is known already: Abnormal germline DNA methylation has been proposed as possible mechanism compromising spermatogenesis in some men with IMI. Dietary folate influence epigenetic modification/s during spermatogenesis. Recently, associations between four SNPs (MTHFR C677T, MTHFR A1298C, MS A2756G and MTRR A66G) in male infertility are widely studied among several ethnicities; however, results remaining contradictory. Folic acid supplementation usually been given to infertile men, a time when DNA methylation patterns are being actively maintained and remodeled in male germ cells to prepare the epigenome for embryogenesis. It is therefore crucial to understand the possible impact of antioxidant with folic acid supplementation in IMI.

Study design, size, duration: Prospective study; azoospermic/ oligozoospermic men with IMI (n=102; Group A) served as study cohort. Normozoospermic men (WHO criteria, 2010) were treated as controls (n=58; Group B). Patients are supplemented with folic acid (5mg/day) and a combination of antioxidant/s (glutathione: 400 mg/day; vitamin E: 200mg/day) for 3 months. Peripheral blood samples are collected before and after treatment. The study was conducted from October 2018 to September 2019 and approved by Institutional Ethics Committee of the Institute.

Participants/materials, setting, methods: Plasma levels of homocysteine, vitamin B12, folate are determined by chemiluminescence. PCR-RFLP (polymerase chain reaction- restriction fragment length polymorphism) and Sanger sequencing by MiSeq(Illumina) was performed to detect SNPs in homocysteine metabolism (MTHFR 677C>T; MTHFR1298A>C; MS2756A>G). The effect of drug supplementation on sperm parameters and apoptosis status was evaluated by Makler's chamber and flow cytometry respectively. Statistical significance was set at $p < 0.05$ as evaluated by student's T-test.

Main results and the role of chance: In comparison with normozoospermic spermatozoa, there was a significant decrease ($P < 0.03$) in motility and velocity parameters and increase in Caspase+/propidium iodide- (PI-) cells ($P < 0.04$) in oligospermic patients. Increased ($p<0.01$) serum homocysteine levels (mmol/L) was documented in Group A with no other changes in blood profile. MTHFR 1298A>C gene polymorphism was documented in 49/102 (48.03%) in contrast to 11 samples from Group B. 7 participants (14.28%) were homozygous for the MTHFR TT variant while 13 (26.53%) and 10 (20.41%) were CC and CT genotypes, respectively (Group A). 29 cases exhibited MS 2756A>G polymorphism in contrast to Group B (n=11). Serum folate concentrations (nmol/L) increased significantly (28 + 2.72 vs. 42 + 3.81; $p<0.01$) after supplementation with no significant improvements in the three MTHFR genotypes for any of the semen parameters (count, motility). Changes in semen volume, sperm motility and sperm chromatin integrity (assessed by sperm chromatin structure assay or SCSA, and reported as % high DNA stainability) was not significant. Sperm DNA integrity, assessed by SCSA and reported as % DNA fragmentation index (% DFI), demonstrated a trend toward improvement (t-test $P = 0.057$) in oligospermic men after combination regimen.

Limitations, reasons for caution: The global erasure of parental DNA methylation during early stages of embryogenesis is a mechanism for reducing the risk of altered methylation. Thus, recommendations for men with IMI to receive combination of antioxidant-folic acid therapy should be taken with caution..

Wider implications of the findings: A personalized approach to combination of antioxidant-folic acid therapy may be needed, based on factors like genetic background of the recipients. Use of combination treatment did not significantly improve semen quality probably because altered methylation may elude global erasure mechanisms and limit the progress to next stages of mitochondrial development.

Trial registration number: NA

P-090 Sperm selection using microfluidic sorting chips in patients with high DNA fragmentation improves clinical outcomes in egg-donor cycles

A. Farreras Ayestaran[1], A. Munuera[1], B. Freijomil[1], S. Novo[1], À. Garcia-Faura[1], B. Marquès[1], F. Garcia[1], C. Castelló[1], M. Lopez-Teijón[1]

[1]Institut Marques, Reproductive Medicine Service, Barcelona, Spain

Study question: Can sperm selection using microfluidic sorting chips improve reproductive outcomes in patients with high sperm DNA fragmentation (SDF)?

Summary answer: Microfluidics sperm selection significantly increases the number of blastocysts and improves clinical pregnancy rate in egg-donation (ED) cycles and high SDF.

What is known already: Previous studies have described a DNA fragmentation negative effect on early embryo development. Concretely, high levels of DNA fragmentation have been associated with embryo development arrest, as well as low implantation and pregnancy rates. Conventional methods used to select sperm for ICSI, as swim-up or density gradients, require centrifugation. It is well known that centrifugation forces increase Reactive Oxygen Species (ROS), one of the main promoter of SDF. Microfluidic systems don't need centrifugation, consequently avoid ROS formation and could allow us to select sperm with better motility, morphology and lower SDF than conventional sperm selection methods.

Study design, size, duration: Preliminary retrospective cohort study including until now 63 cycles of ICSI-ED and sperm with high SDF (>30%) between January 2017 and October 2019. In all the cycles single fresh blastocyst transfer were performed. SDF was evaluated by SCD (Sperm Chromatin Dispersion) test.

Participants/materials, setting, methods: Two groups of cycles were established according to the sperm selection method used for ICSI: microfluidic chip (G1; n=30) and density gradients (G2; n=33). The groups were homogeneous in terms of the donor age, mean number of mature oocytes collected and embryos transferred. No differences were found between groups in the SCD test using T-student statistics (G1: 45.6±14.5%; G2: 39.0±11.7%; $p=0.05$). The results were analyzed using Fisher test.

Main results and the role of chance: The two considered groups of this study were diagnosed with pathological percentage of sperm DNA fragmentation. Fertilization rate was equivalent ($p=0.07$) between G1 (172/216; 79.6%) and G2 (169/234; 72.2%). However, good quality blastocyst rate (GQB; ≥3BB Gardner score) of G1 (84/172; 48.8%) was significantly higher ($p<0.05$) than in G2 (64/169; 37.9%), as well as pregnancy rate (G1: 90.0%; G2: 54.55%). Additionally, clinical pregnancy rates were higher in G1 (63.3%) than in G2 (48.5%), not being statistically significant. Perhaps, with a higher sample could help to asses if the tendency of this increase keeps on the clinical pregnancy rate. Our results show that processing sperm samples with a microfluidic sperm sorting device could be a more efficient alternative to density gradients in cycles where patients were diagnosed with increased SDF. This method allows the selection of sperm with a low DNA fragmentation promoting the formation of a higher number of GQB to transfer and/or vitrify.

Limitations, reasons for caution: This is a preliminary retrospective study, so the main limitation is the small sample size achieved until now. These results should also be confirmed by further randomized prospective studies.

Wider implications of the findings: Our findings suggest that the application of microfluidic systems could be improving the sperm selection with conserved DNA integrity. In this sense, microfluidic systems increase significantly the number of GQB and therefore could potentially improve the cumulative pregnancy rates and the efficiency of the cycle.

Trial registration number: not applicable

P-091 Fertile®ChiP-ZyMōt improves ICSI clinical outcomes in patients with high values of sperm double-strand breaks using oocytes from both patients and donors.

S. Camacho Fernandez-Pacheco[1], C. Urda-Muñoz[1], S. Lara-Cerrillo[2], T. Lacruz-Ruiz[2], C. Rosado-Iglesias[2], M. De la Casa Heras[1], V. Badajoz-Liébano[1], J. Gijón de la Santa[1], A. García-Peiró[2]

[1]GINEFIV, Assisted Reproduction, Madrid, Spain ;
[2]CIMAB- Barcelona Male Infertility Centre, Research & Development, Sant Quirze del Vallés - Barcelona, Spain

Study question: Are clinical outcomes on ICSI cycles improved by the use of Fertile®ChiP-ZyMōt for sperm selection using oocytes from both patients and donors?

Summary answer: Fertile®ChiP-ZyMōt improved biochemical and clinical pregnancy rates and reduced miscarriage rate in ICSI cycles from patients with altered values of dsSDF.

What is known already: Delays in embryo kinetics, implantation failures in ICSI treatments and recurrent miscarriages have been associated to high values

of double-strand breaks (DSB) in sperm. Moreover, a recent study showed that DSB are not reduced during the sperm selection of an ICSI cycle.

The Fertile®ChiP-ZyMōt is a new method for sperm selection based on microfluidic properties that showed to reduce the presence of DSB in the sperm sample. In this sense, the specific reduction of DSB using Fertile®ChiP-ZyMōt could improve clinical outcomes after ICSI treatments.

Study design, size, duration: This retrospective study included 78 ICSI cycles from January 2018 to March 2019. Three groups were classified attending on the origin of the oocytes and the sperm selection method: Control group = oocytes from patients and density gradients for sperm selection (n=16); Group 1 = oocytes from patients and Fertile®ChiP-ZyMōt for sperm selection (n=22); and Group 2 = oocytes from donors and Fertile®ChiP-ZyMōt for sperm selection (n=11). All male patients presented high values of DSB.

Participants/materials, setting, methods: Patients included in the study presented high values of DSB analyzed though the Neutral CometFertility assay (CIMAB, Spain). Sperm selection was performed using conventional Density Gradients (Sperm Grad, Vitrolife, Sweden) (Control group) or the Fertile®ChiP-ZyMōt (DxNow, USA) (Groups 1 and 2). ICSI cycles were performed using oocytes from patients or donors and clinical outcomes were studied. Results were compared between groups, being the statistical significance $\alpha = 0,05$.

Main results and the role of chance: Women's age was significantly lower in Group 2 (26.7 ± 4.28) compared to the Control group (35.67 ± 3.43) and Group 1 (36.17 ± 3.84), $p<0.01$.

Fecundation rates were slightly higher, even not significantly, in Group 1 (0.53 ± 0.27) and Group 2 (0.63 ± 0.21) compared to the Control group (0.51 ± 0.29), $p=0.80$. Biochemical pregnancy was significantly higher in Group 1 (12/22 (54.5%)) and Group 2 (7/11 (63.6%)), compared to the Control group (3/16 (18.8%), $p=0.03$. Clinical pregnancy was significantly higher in Group 1 (10/22 (45.5%)) and Group 2 (7/11 (63.6%)), compared to the Control group (2/16 (12.5%), $p=0.01$. Miscarriage rates were significantly higher in the Control group (2/2 (100%)) than in Group 1 (2/10 (20%)) and Group 2 (0/7 (0%)), $p=0.02$.

Even Group 2 showed the best results, there were no significant differences compared to Group 1 for biochemical and clinical pregnancies and miscarriage rates ($p=0.618$; $p=0.325$ and $p=0.418$, respectively).

Compared to density gradients, the use of the Fertile®ChiP-ZyMōt in ICSI cycles improved biochemical pregnancy rates x1,65 using oocytes from patients ($p=0,033$) and x2,32 using oocytes from donors ($p=0,054$). Fertile®ChiP-ZyMōt also improved clinical pregnancy rates x1,71 using oocytes from patients and x2,68 oocytes from donors ($p=0,036$).

Limitations, reasons for caution: Despite biochemical and clinical pregnancies presented significant better results using the Fertile®ChiP-ZyMōt, the increase in the fecundation rate was not significant. More studies analysing a larger number of ICSI cycles are needed to confirm these findings.

Wider implications of the findings: The use of the Fertile®ChiP-ZyMōt in ICSI cycles to treat high values of DSB in sperm increase biochemical and clinical pregnancy rates. These increases are even more important when oocytes are from a donor.

Trial registration number: Does not apply.

P-092 Varicocelectomy corrects sperm capacitation functions and enhances sperm genomic integrity

M. Haddad[1], P. Xie[1], A. Parrella[1], Z. Rosenwaks[1], J. Kashanian[2], G. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A. ;
[2]Weill Cornell Medicine, Department of Urology, New York, U.S.A.

Study question: To determine whether varicocelectomy improves spermatogenesis by enhancing semen parameters, sperm capacitation, sperm genomic integrity, and overall embryo developmental competence of the male gamete.

Summary answer: Correction of grade 2 or higher varicocele enhanced male gamete production, ameliorated sperm chromatin fragmentation (SCF), and amplified male gamete capacitation.

What is known already: Varicocele is known to induce oxidative damage to the male reproductive system, affecting semen parameters, sperm chromatin integrity, and function. Varicocelectomy has been proposed to enhance typical parameters measured in semen analysis, such as concentration and motility, and ameliorate SCF. Because the predictability of overall gamete competence through semen analysis has been debatable, new bioassays have been proposed. By surveying localization patterns of ganglioside M_1, a key regulator of capacitation and acrosomal reaction, we have been able to quantify subtle sperm function and predict probability of generating a pregnancy (PGP).

Study design, size, duration: In the past 12 months, fresh ejaculates were obtained from 6 consenting men who were initially diagnosed with varicocele of grade 2 or higher and then treated by varicocelectomy. Ejaculates were obtained again at least 3 months post-surgery to allow a full cycle of spermatogenesis. Semen parameters, SCF, CaP-Score™, and PGP were determined in a blind fashion and compared among pre- and post-operative specimens.

Participants/materials, setting, methods: Semen analysis was performed on pre-/post-operative ejaculates. Varicocele was evaluated by physical exam in standing position. Capacitation was measured by CaP-Score™ (Androvia LifeSciences) with normal thresholds of >27.6%. A corresponding PGP was previously established by analyzing pregnancy outcomes from fertile and infertile men who completed 3 IUI cycles with a normal threshold of >32.7%. Total capacitated spermatozoa were quantified by volume x concentration x CaP-ScoreÔ. SCF was assessed using TUNEL with normal threshold of <15%.

Main results and the role of chance: Men (n=6) with grade 2 varicocele or higher aiming to procreate underwent microsurgical varicocelectomy without any post-operative complication. Semen parameters such as volume and normal morphology that were assessed before surgery did not improve. For all men, sperm concentration was initially 35.0 ± 28.0 x10^6/ml and became 57.3 ± 29.5 x10^6/ml ($P < 0.05$). Sperm capacitation function, total capacitated spermatozoa, and PGP at baseline were 25.4 ± 3.4%, 18.4 ± 13.3 x10^6, and 29.3 ± 5.1%, respectively. After 3 months of post-operative recovery, sperm capacitation, number of total capacitated spermatozoa, and PGP significantly increased to 32.0 ± 4.1%, 33.2 ± 16.0 x10^6, and 41.0 ± 7.2% ($P < 0.05$), respectively. Genomic integrity as measured by SCF was originally above threshold at 17.9 ± 6.6% and significantly decreased to an average of 11.0 ± 4.8% ($P < 0.01$) after varicocele correction.

Limitations, reasons for caution: These findings support a beneficial effect of varicocele correction. However, the improvement of sperm concentration together with functional assay, as well as the amelioration of genomic integrity, needs to be confirmed by testing the embryonic developmental competence of the male gamete.

Wider implications of the findings: This analysis confirms the beneficial effect of varicocelectomy on at least one semen parameter. The utilization of a functional assay and the determination of genomic integrity by SCF provide information on an individual's ability to spontaneously reproduce or can help guide one toward the preferential method of assisted reproduction.

Trial registration number: not applicable

P-093 Andropenia precedes AML diagnosis and is associated with fatty marrow

K. Shlush[1], N. Zioni[2], N. Kaushansky[3], G. Oron[4], L. Shlush[3]

[1]fertility clinic Rabin Medical center, Obstetric and Gynecology, Herzliy, Israel ;
[2]Wiezmann institute of science, Immunology, Rehovot, Israel ;
[3]Weizmann institute of science, Immunology, Rehovot, Israel ; [4]Rabin medical center, Obgyn, Petah Tikva, Israel

Study question: What are the effects of andropenia on leukemia and bone marrow?

Summary answer: Many years before AML diagnosis testosterone levels are decreasing. Low testosterone levels after castration cause fatty bone marrow in mice.

What is known already: As human age they accumulate somatic mutations. These early mutations preleukemic mutations (pLMs) accumulate and eventually lead to different myeloid malignancies. Specific pLMs are more common among elderly males. Furthermore myeloid malignancies are more common among males. While large proportion of the population carry pLMs only a small proportion will develop myeloid malignancies. Understanding the mechanisms contributing to the progression to leukemia is of great importance. One factor possibly contributing to leukemia evolution might be the accumulation of fat in the bone marrow (BM) with age. A correlation was found between increased BM fat and low testosterone levels.

Study design, size, duration: The electronic health records of 4.5 million Israelis over 15 years were analyzed to identify AML cases (N=987). We identified all individuals in this group (pre-AML) and studied their blood counts and testosterone levels before AML diagnosis and compared it to 500,000 aged match individuals.
Participants/materials, setting, methods: To study the effect of andropenia on BM fat mice were castrated and BM fat was measured after two month by lipitox staining of CD45 negative cells from the BM. in each group 10 mice were used.
Main results and the role of chance: Testosterone levels were available for 76 pre-AML cases on average 4 years before AML diagnosis. Significantly reduced testosterone levels was present already four years before AML diagnosis and remained significantly lower until diagnosis. Low testosterone levels were achived after castration. Two month after castration a significant increase in the BM fat was noticed with 30% more fat in the castrated mice compared to aged matched controls.
Limitations, reasons for caution: It is not clear why fatty marrow is promoting leukemia among males. Also the number of pre-AML cases with testosterone levels was low and a sampling bias is possible.
Wider implications of the findings: The effect of low testosterone levels on the BM and leukemia evolution remains unknown.In the current study we provide evidence that fatty marrow can be induced by castration. Furthermore, low testosterone levels precede AML. Better understanding the mechanisms leading to increased leukemia might help in preventing this devastating disease.
Trial registration number: NA

P-094 The impact of double-stranded DNA breaks in human spermatozoa on embryo implantation

L. Zimmerman[1], D. Keating[1], A. Parrella[1], Z. Rosenwaks[1], G. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Is there a correlation between double-stranded DNA breaks (DSBs) in human spermatozoa and ICSI outcome?
Summary answer: An increased proportion of DSBs in men with normal semen analyses impairs embryo implantation.
What is known already: Total sperm chromatin fragmentation (SCF) in men with normal semen parameters has been linked to a couple's ability to successfully conceive. Recent studies have challenged this, asserting that DSBs, rather than single-stranded breaks (SSBs), lead to compromised embryo development. There are many techniques to assess the genomic integrity of spermatozoa by measuring indistinct SSBs and DSBs, such as the sperm chromatin structure assay, terminal deoxynucleotidyl dUTP transferase nick-end labeling (TUNEL), sperm chromatin dispersion, and alkaline Comet. However, the only method to specifically detect double-stranded DNA integrity is the neutral Comet test.
Study design, size, duration: In the course of 12 months, we carried out a pilot study to assess exclusively DSBs in spermatozoa by neutral Comet assay. There was a linear correlation between Comet and TUNEL (R2=0.96), which allowed us to extrapolate the proportion of DSBs in all men who had been screened by TUNEL assay. This value was correlated with ICSI outcome.
Participants/materials, setting, methods: A total of 523 men with poor assisted reproductive outcomes were screened by TUNEL, with ≥500 spermatozoa assessed per sample and a normal threshold of ≤15%. Neutral comet was performed using a modified in-house protocol with commercially available materials, with ≥200 spermatozoa assessed, and a normal threshold of ≤3%. All men underwent ICSI, performed in the standard fashion, with their female partners. Fertilization, implantation, and pregnancy outcomes were compared according to DSBs.
Main results and the role of chance: The pilot study yielded an average SCF by TUNEL of 11.3±6% and a DSB of 2.2±3% by neutral Comet in a linear relationship (R2=0.96). This allowed us to extrapolate DSB rates in 523 normozoospermic men (2.6±1 mL volume, 42.3±34x106 concentration, 43.1±10% motility, and 4.1±1% normal morphology) who had undergone TUNEL.

A total of 377 couples with normal DSB rates underwent 736 ICSI cycles. The average maternal age was 37.0±4 years, and the paternal age was 38.5±6 years. These cycles had a 72.7% fertilization rate (4935/6789), with a 12.0% implantation rate (112/935) and a 21.7% clinical pregnancy rate (CPR; 92/423). Fourteen of these pregnancies resulted in miscarriage (15.2%).

A total of 146 couples with an abnormal DSB level were treated by 259 ICSI cycles. The maternal age was comparable between the two groups at 37.3±5 years, while male partners were much older at 41.4±8 years of age (P<0.01). This group had a comparable fertilization rate of 70.1% (1739/2481), an implantation rate of 10.8% (49/455), and a CPR of 22.9% (40/175). However, these couples were significantly more likely to experience a miscarriage, which occurred at a rate of 30.0% (12/40; P<0.05).
Limitations, reasons for caution: This is a retrospective observation in couples with a history of compromised outcomes with assisted reproductive techniques. It would be ideal to reproduce these findings in a prospective manner using solely the neutral Comet assay.
Wider implications of the findings: DSBs in human spermatozoa, which have been associated with structural chromosomal abnormalities, may provide information on embryonic developmental potential. Utilizing an assay that screens exclusively for DSBs may be more beneficial than total SCF in terms of predicting a couple's ability to successfully reproduce.
Trial registration number: not applicable

P-095 The male factor – impact of semen quality on the insemination result in women with endometriosis

Z. Kaszás[1], A. Nemes[1], Á. Anikó[1], D. Pócsi[1], Á. Murber[1], J. Urbancsek[1], P. Fancsovits[1]

[1]Division of Assisted Reproduction Semmelweis University, Department of Gynecology and Obstetrics, Budapest, Hungary

Study question: Do we need higher sperm count to achieve a successful insemination cycle with endometriosis patients?
Summary answer: Our results suggest that sperm viability and/or its lifetime is effected by endometriosis.
What is known already: Little is known about the effect of endometriosis on the outcome of insemination (IUI). In the first 6 month after surgery women with minimum to mild endometriosis have the same chance to get pregnant with IUI as couples with unknown infertility. The ESHRE guideline recommend insemination as treatment for infertility with minimum to mild endometriosis, but this statement has never been linked to any study with male fertility factors. We don't know yet whether sperm react to the intrauterine environment effected by endometriosis, but possibly endometriosis may influence the viability of the motile sperm.
Study design, size, duration: This is a retrospective study performed between 2010 January and 2019 May at university settings. Patients treated with endometriosis and infertility were selected from our institute database such as diagnosis of endometriosis, date of previous surgeries, date of insemination and total motile sperm count (M/ml) after density gradient centrifugation.
Participants/materials, setting, methods: Data of IUI treatments were analyzed in two groups. Endometriosis group: cycles with the diagnosis of previously surgically proven endometriosis. Control group: cycles with "unknown infertility".

Endometriosis group was further divided into two subgroups according to the time elapsed between surgery and IUI. Subgroup A: IUI ≤6 months after surgery; Subgroup B: IUI in 7-18 months after surgery.

Total motile sperm count and pregnancy rates were compared in Endometriosis and Control groups.
Main results and the role of chance: The pregnancy rate of insemination cycles of women with endometriosis (23/341; 6,7%) was similar to the Control group (9/171;5,2%) (P=0,513).

Subgroup analysis of endometriosis patients showed similar pregnancy rates in Subgroup A (4/69; 5,8%) and Subgroup B (10/129;7,7%) (P=0,609).

The progressive motile sperm count in the pregnant IUI cycles was 34,9± 18,4 M/ml, in the Endometriosis group, while 25,8±14,6 M/ml in the Control group. The difference was not significant (P=0,304).

The mean of 22,5 ±9,2 M/ml of progressive sperm count was found in pregnancy in Subgroup A. That is similar to the Control group's (25,8± 14,6 M/ml) result.

However, that of Subgroup B higher sperm number was found in pregnancy cycles (37,3 ±11,3 M/ml), but the difference was not significant (P=0,327) compared to the Control group.

It seems that within 6 months following the endometriosis surgery patients need comparable amount of motile sperm to get pregnant by insemination as the patients with unknown infertility. After this half year period, patients need higher sperm count to get pregnant. These differences can be explained with the recurrent appear of endometriosis changing the pelvic environment.
Limitations, reasons for caution: The retrospective study design limits our chance to make a strong conclusion. Our small sample number in the subgroups also limits our chance to find significant differences.
Wider implications of the findings: Our results could also confirm the ESHRE guideline regarding the necessity of insemination during the first 6 months after the endometriosis surgery. We plan to further explore this field, to find out what kind of biochemical milieu could influence the need for higher sperm count to reach pregnancy.
Trial registration number: not applicable

P-096 Increased DNA damage in infertile patients with high grading varicocele

S. Jellad[1], F. Hammami[1], A. Khalbous[2], M. Cbibani[3], R. Rachdi[3]

[1]Miitary Hospital of Tunis- Faculty of Medicine of Tunis, laboratory of Reproductive Biology- Unit of Assisted Medical Procreation, Tunis, Tunisia ;
[2]Miitary Hospital of Tunis, laboratory of Reproductive Biology- Unit of Assisted Medical Procreation, Tunis, Tunisia ;
[3]Miitary Hospital of Tunis- Faculty of Medicine of Tunis, Unit of Assisted Medical Procreation, Tunis, Tunisia

Study question: This study aims' at evaluating the impact of clinical varicocele on sperm nuclear DNA quality and standard semen parameters?
Summary answer: Clinical varicocele generates a significant increase of sperm abnormalities and DNA damage and these changes are positively correlated with varicocele grade.
What is known already: Varicocele is thought to progressively reduce spermatogenesis via elevated intratesticular temperature and altered testicular blood flow. The decreased supply of oxygenated blood and nutrients to the sperm secretion sites reduces sperm quality and quantity, and consequently, their fertility capacity. Although a cause-effect relationship is not established, multiple reviews conclude that there is indeed evident association between varicocele and increased DNA fragmentation
Study design, size, duration: The study included 45 men assessed by our laboratory of reproductive biology of military hospital of Tunis during 6 months. A prospective study was designed involving one control group of men with unknown fertility and normal semen parameters (n = 10) and one group of patients with clinically diagnosed varicocele and infertility (n=30).
Participants/materials, setting, methods: Our prospective study involved 30 infertile patients with clinical varicocele and 15 control patients referred to our laboratory for routine spermiological exploration). Men with azoospermia, severe oligozoospermia or leucocytospermia were excluded from the study. Spermograms were performed and analyzed according to WHO guidelines 2010. The DNA fragmentation was detected by the terminal desoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick-end labeling (TUNEL) assay.
Main results and the role of chance: The average age of the patients at the time of diagnosis was 26 ± 4 years (19-35) and 35 ± 7 years (27-47) for the control group. The DNA fragmentation index was significantly higher in patients with clinical varicocele compared to controls (13.3±3.4 % versus 6.1±2.5%, p=0.0001). In addition, the DFI was positively and significantly correlated with the degree of severity of varicocele thus the DFI was 15.24±1.9% in patients with grade 3 versus 12.92±3.5 % in those with grade 2 (p<0.0001). However, an abnormality of at least one of the semen parameters was found in 90% of varicocele patients, and all semen characteristics such as sperm count, vitality, mobility and typical forms were decreased compared to the controls. Furthermore, statistically significant negative correlations were noted between sperm DNA fragmentation index and sperm concentration (p=0.0001), motility (p=0.03), and normal sperm morphology (p=0.03).
Limitations, reasons for caution: Although considered as referent technique the TUNEL assay has certain limits as it doesn't allow differentiating between normal and pathological DNA ruptures. Further investigations are also needed to reveal the cause and effect relationship between the increase of DNA fragmentation levels and clinical varicocele, as well as the underlying mechanisms.
Wider implications of the findings: Sperm DNA damage independent of its cause, may affect the sperm quality and have implications on patient's fertility potential. Therefore, we recommended the evaluation of sperm DNA status in infertile patients with a high clinical varicocele grade in order to optimize sperm quality and pregnancies rates in this population.
Trial registration number: not applicable

P-097 Male lifestyle and pathological conditions associated with couple infertility in a referral center in Italy: a cross-sectional study of 13452 male patients

M. Dal Canto[1], C. Signorini[2], L. Mura[1], S. Casellato[3], L. Carmignani[2], M.M. Renzini[1], J. Buratini[1,4], S. Maruccia[3]

[1]Biogenesi - Reproductive Medicine Centre, Istituti Clinici Zucchi, Monza, Italy ;
[2]U.O. Urologia IRCSS Policlinico San Donato, Milano, Italy ;
[3]U.O. Urologia Istituti Clinici Zucchi, Monza, Italy ;
[4]Sao Paulo State University, Institute of Biosciences, Department of Physiology

Study question: Is overall male health associated with male fertility as assessed by semen analysis in couples undergoing subfertility treatment?
Summary answer: Oncologic, cardiovascular, metabolic and allergic diseases are more frequent in infertile men with impaired semen analysis than in those with normal semen parameters.
What is known already: Current literature suggests an association between male infertility and a wide range of other comorbid conditions, ranging from oncologic, cardiovascular, autoimmune, and metabolic disorders. The exact nature of this putative association remains somewhat unclear, although hypothesized mechanisms linking men's health and fertility include genetic, developmental, and lifestyle-based factors. The male population seeking for fertility treatment constitutes an extremely valuable opportunity to clarify this issue, but have not been sufficiently explored in previous studies with this aim.
Study design, size, duration: Sociodemographic, health, lifestyle and recreational data from 13452 male patients seeking for couple infertility treatment were collected at a single referral centre between 01/2013 and 12/2019. Frequencies of diseases and lifestyle/recreational habits impacting on health (smoking, use of alcohol and other drugs of abuse) were compared between male patients with different fertility as assessed by semen analysis.
Participants/materials, setting, methods: This is a retrospective study utilizing data collected from 13452 male partners of couples seeking for infertility treatment at our clinic. Treatments included 1091 IUI, 7443 ICSI and 1348 IVF. The WHO 2010 referral criteria were used to categorized semen parameters as pathological or normal. Descriptive statistics was applied to describe the whole cohort. Differences in frequencies were assessed with the Chi Square test.
Main results and the role of chance: The median age was 41.5 years (95% CI ±0,09). 8209 (61.0%) men had pathological semen parameters (any type), while 5243 (39.0%) individuals presented normal semen parameters. Among patients with pathological semen, 3666 (44.6%) had a sperm concentration <15mln/ml, 3689 (44.9%) had a total sperm count <39 mln, 4695 (57.1%) had a motility <32%, and 6764 (82,3%) men had a normal morphology <4%. In terms of health comorbidities, patients with abnormal semen parameters more frequently reported a history of hypertension [941 (11.4%) vs. 234 (4.4%)], diabetes mellitus [804 (9.7%) vs. 178 (3.3%)] and cardiovascular diseases [613 (7.4%) vs. 160 (3.0%)], than those with normal semen analysis (p<0.001). Testis cancer was more frequently found in men with abnormal semen parameters [140 (1.7%) vs. 24 (0,4%), p<0.001]. Men with impaired semen parameters were more frequently active smokers [3336 (40.6%) vs. 764 (14.5%)] and alcohol consumers [208 (2.5%) vs. 15 (0.2%)] than those with normal semen parameters (all p<0.001), while the use of substances of abuse was similar among groups. A history of allergic diseases was more frequently found in men with impaired semen analysis [2031 (24.7%) vs. 477 (9.0%); p<0.001].
Limitations, reasons for caution: The retrospective nature of the study.
Wider implications of the findings: Our data contribute to a better comprehension of the association between men's health and fertility, while favouring early diagnosis of important male diseases and medical counselling during subfertility treatment.
Trial registration number: Not applicable

P-098 Improved classification of testicular histopathology to promote uniform diagnosis and discovery of genetic causes for male infertility

G. Van der Heijden[1], M. Wyrwoll[2], L. Ramos[1], J. Veltman[3], F. Tüttelmann[4]

[1]Radboud University Medical Center, Gynaecology, Nijmegen, The Netherlands ; [2]University of Münster, Department of Clinical and Surgical Andrology- Centre of Reproductive Medicine and Andrology, Münster, Germany ; [3]Newcastle University, Institute of Genetic Medicine, Newcastle, United Kingdom ; [4]University of Münster, Institute of Human Genetics, Münster, Germany

Study question: Can a descriptive framework for testicular histopathology be designed that identifies phenotypically unique cases and facilitates research on the genetic causes of male infertility?

Summary answer: We propose an organized nomenclature for testis histopathology that makes use of clearly defined rules and definitions, describing certain patterns of disruption of spermatogenesis.

What is known already: With the availability of Next Generation Sequencing the genetic causes of male infertility are increasingly being chartered. A genetic diagnosis combined with a clear unambiguous description of accompanying testis histology will hugely benefit interpretation of the function of identified genes. However, a universal ontology for testis histopathology is lacking. The Human Phenotype Ontology (HPO) is a formal ontology, which describes phenotypes encountered in human diseases. The HPO allows phenotype driven differential diagnostics in clinical routine and research. The current hierarchy of terms regarding azoospermia, is not a systematical one. Moreover, the majority of testicular phenotypes are not annotated yet.

Study design, size, duration: We aimed to design a framework that is easy to understand and straightforward in its application but does contain most relevant information to study the impact of an identified mutation on spermatogenesis.

Participants/materials, setting, methods: The ontology classification is based on qualitative and quantitative histology description of testicular samples from azoospermic samples (>1000) in our centers. For the differentiation between the classification of hypospermatogenesis and complete spermatogenesis, n=117 testicular biopsies post vasectomy cases were used.

Main results and the role of chance: Building on what has been published previously we propose a framework for testis histopathology based on the presence/absence of germ cells and/or Sertoli cells. Our framework has five main categories. In addition to Sertoli cell only phenotype we add the classification of tubular shadows, when also Sertoli cells are absent. When germ cells are present, their stage of arrest, numbers and distribution can inform us about the effect of the causal mutation. We therefore propose to distinguish Germ Cell Arrest (GCA), in which no spermatozoa are observed, from hypospermatogenesis (HS). Both categories are sub-divided to obtain information on the timing of the arrest (GCA) or the severity of overall loss of spermatogenesis (HS). To establish a lower threshold for complete spermatogenesis we determined the number of tubules that contain elongating spermatids (ES) in a collection of samples of men with an obstructive azoospermia due to a previous vasectomy (N=117). The majority of samples contained ES in ≥60% of their tubules, suggesting 50% to be a reasonable cut-off to distinguish hypospermatogenesis from complete spermatogenesis. We conclude that once this framework is incorporated in the HPO, the standardized vocabulary will facilitate communication and interpretation in both a clinical routine and research.

Limitations, reasons for caution: Words describing the world are words not the world. Some classifications of histopathology remain arbitrary. Discussions should follow to further improve categories and reach a consensus.

Wider implications of the findings: Histopathology of the testis is just one aspect of the diagnosis, treatment and study of male infertility. The use a uniform ontology for all clinical diagnoses concerning male infertility should be facilitated and stimulated.

Trial registration number: not applicable

P-099 A randomized clinical trial comparing intracervical insemination and intrauterine insemination for donor sperm treatment in the natural cycle.

F. Kop[1], M. Van Wely[1], A. De Melker[1], B.W. Mol[2], R. Bernardus[3], M. De Brucker[4], P. Janssens[5], A. Nap[6], B. Cohlen[7], J. Pieters[8], S. Repping[1], F. Van der Veen[1], M. Mochtar[1]

[1]Amsterdam UMC- University of Amsterdam, Centre for Reproductive Medicine-Amsterdam Reproduction & Development Institute, Amsterdam, The Netherlands ; [2]Monash University, Department of Obstetrics and Gynaecology, Melbourne, Australia ; [3]Nij Barrahûs, Fertility clinic, Wolvega, The Netherlands ; [4]University hospital Brussels, Centre for Reproductive Medicine, Brussels, Belgium ; [5]Rijnstate, Department of Clinical chemistry and haematology, Arnhem, The Netherlands ; [6]Rijnstate, Department of Obstetrics and Gynaecology, Arnhem, The Netherlands ; [7]Isala, Department of Obstetrics and Gynaecology, Zwolle, The Netherlands ; [8]Vivaneo Medisch Centrum Kinderwens, Fertility Clinic, Leiderdorp, The Netherlands

Study question: Are six cycles of ICI non-inferior to six cycles of IUI in donor sperm treatment in the natural cycle in terms of ongoing pregnancy?

Summary answer: In donor sperm treatment in the natural cycle in terms of ongoing pregnancy rate, we could not demonstrate non-inferiority of ICI as compared to IUI.

What is known already: Both ICI and IUI in the natural cycle are performed as first line treatments in women who are eligible for donor sperm treatment. IUI is more costly than ICI, due to the involvement of sperm processing. High quality data on the effectiveness of ICI versus IUI in the natural cycle in terms of ongoing pregnancy are lacking. A large retrospective cohort study performed in the Netherlands suggested that ICI and IUI in the natural cycle in donor sperm treatment resulted in comparable ongoing pregnancy rates of 40% after six cycles.

Study design, size, duration: We performed a multicenter, non-blinded, non-inferiority randomized controlled trial in six fertility clinics in the Netherlands and Belgium. Women were allocated to receive either ICI or IUI in a natural cycle during six cycles. Based on the retrospective cohort study we assumed a live birth rate of 40% after six cycles of IUI. To assess a non-inferiority margin of 12%, we needed to include 416 women.

Participants/materials, setting, methods: All women scheduled for donor sperm treatment were eligible, regardless of the indication for treatment. The primary outcome was ongoing pregnancy within eight months after randomisation leading to a live birth. Secondary outcomes were multiple pregnancy, miscarriage, time to ongoing pregnancy and pregnancy complications.

We calculated relative risks (RR) and risk difference (RD) and 95% CI. We analysed the data both on an intention to treat and per protocol basis.

Main results and the role of chance: Between June 2014 and February 2019, we included 419 women, of whom 211 women were randomly allocated to ICI and 208 to IUI.

Women's age was on average 34 years (SD ± 4) in both groups.

Of the 419 women included, ongoing pregnancy occurred in 52 women (25%) in the ICI group and 77 women (37%) in the IUI group (RR 0.67, 95% CI 0.50 to 0.89 and RD -0.12, 95% CI -0.21 to -0.04). Live birth occurred in 51 women (25%) in the ICI group and 76 women (37%) in the IUI group (RR 0.67, 95% CI 0.50 to 0.91).

In the per protocol analysis we included 193 women in the ICI group and 193 women in the IUI group. We excluded two spontaneous pregnancies. Ongoing pregnancy occurred in 51 women (26%) in the ICI group and 76 women (39%) in the IUI group (RR 0.67,95% CI 0.50 to 0.90 and RD -0.12, 95% CI -0.21 to -0.03). In both the intention to treat and per protocol analysis ICI was inferior to IUI as the left boundary of the 95% confidence interval crossed the pre-set absolute difference of 12%.

Limitations, reasons for caution: We did not perform analyses for factors possibly affecting ongoing pregnancy rate like quality of frozen-thawed donor sperm. These data will be available in July 2020.

Wider implications of the findings: In women undergoing donor sperm treatment in a natural cycle, ICI results in lower ongoing pregnancy rates than IUI. Therefore, IUI should be the preferred treatment.

Trial registration number: NTR4462

P-100 Impact of genotype and phenotype on the spermatogenesis of cystic fibrosis (CF) patients: a cohort study about 57 patients from 1998 to 2019

A. Chargui[1], L. Adjiman[1], D. Hubert[2], C. Jean[1], A.S. Gille[1], E. Girodon Boulandet[3], L. Ferreux[1], C. Chalas[1], K. Pocate[1], J.P. Wolf[1], N. Thiounn[4], T. Bienvenu[3], C. Patrat[1]

[1]Hôpital Cochin - Assistance Publique des Hôpitaux de Paris, Service d'Histologie Embryologie Biologie de la Reproduction, PARIS, France ;
[2]Hôpital Cochin - Assistance Publique des Hôpitaux de Paris, Service de Pneumologie - Centre de ressources et de compétences de la mucovisidose, Paris, France ;
[3]Hôpital Cochin - Assistance Publique des Hôpitaux de Paris, Service de Génétique et Biologie moléculaire, Paris, France ;
[4]Hôpital européen Georges Pompidou - Assistance Publique des Hôpitaux de Paris, Service d'Urologie, Paris, France

Study question: Is there an impact of CFTR (cystic fibrosis transmembrane-conductance regulator) gene mutation type and/or general health condition and the quality and yield of spermatogenesis?
Summary answer: Neither genetic severity nor clinical severity seems to have a negative impact on the characteristics of epididymis and testicular spermatozoa ofn cystic fibrosis (CF) patients.
What is known already: CFTR protein regulates electrolyte and fluid transport in many tissues with exocrine function, including male reproductive tract. Mutation of CFTR gene causes CF, which affects the function of several organs, and impairs male fertility. CF is generally associated to an obstructive azoospermia because of bilateral absence of vas deferens and seminal vesicles degeneration. CFTR protein is detected in human fetus at the early developmental stages and highly expressed in both testis and epididymis. CFTR provides establishment of specific fluid environment for germ cell differentiation and maturation. According to literature, it seems likely that CFTR mutations affect the sperm quality.
Study design, size, duration: This cohort study was conducted in the Assisted Reproduction Center of an university hospital on 57 patients, for whom a CF has been diagnosed and monitored at Cystic Fibrosis Resource and Competence Center (CFRCC) in the same hospital. The CF diagnosis was based on CFTR genetic tests and clinical symptoms. All patients were azoospermic and underwent on a microscopic epididymal sperm aspiration (MESA) and/or a testicular sperm extraction (TESE) between 1998 and 2019.
Participants/materials, setting, methods: Clinical data- one year preceding surgical sperm collection - related to the severity of the CF have been collected: respiratory spirometry data, *Pseudomonas aeruginosa* colonization, number of antibiotic treatment, BMI and Pancreatic insufficiency, as well as CFTR mutations. Linear regression tests (fisher and χ^2) were carried out to establish or not correlation between on the one hand the quality of the spermatic sample and on the other hand genotype and/ or each clinical parameter
Main results and the role of chance: The mean age of patients at sperm retrieval was 31.3 years [21-55]. The mean BMI was 21.3 Patients with severe genotype represented 67.8% and likely (67.3%) an external pancreatic deficiency. *P.aeroginosa* colonization was revealed in 30% of cases and 45.6% patients received at least one IV antibiotic treatment a year before MESA and/or TESE. Respiratory function has been impaired in 18 cases (31.6%) with a maximal expiratory volume per second (MAVS) < 40%.

Spermatozoa were founded for all patients and frozen after 58 surgical sperm collections (one patient has benefited of 2 TESE): 20 MESA, 32 MESA + TESE and 6 only TESE. Spermatic parameters results were: presence of epididymal spermatozoa in 89.7%, a mean epididymal sperm numeration at 66.0 ±105.5 million, subnormal mean epididymal vitality at 57.8%. Progressive epididymal motility. Total testicular motility and testicular vitality were decreased (respectively: 2.5±4.7%, 6.0±6.8% and 41.1±25.3%). The mean number of epididymal motile progressive spermatozoa was satisfactory (10.2±18.83 million).

The presence and quality of epididymal spermatozoa was not affected by the genetic severity of the disease (p = 0.65). Likewise, and after several univariate analyses there was no statistically significant correlation between the parameters evaluating clinical severity and those relating to epididymal sperm.
Limitations, reasons for caution: The lack of standard values for epididymal and testicular sperm parameters made harder the choice of cut-offs to establish correlation. This leads us to choose the WHO recommendations for sperm count and vitality and the mediane for motility. Our study needs to be completed by a multivariate analyse.
Wider implications of the findings: Regarding to the better quality of life of CF patients nowadays and to our results- with no significant correlation between genetic and/or clinical severity and spermatogenesis, the surgical sperm collection can be proposed only in case of a conceptional project for men in couple or before transplantation and immunosuppressive agents
Trial registration number: none

P-101 Male Infertility During the Syrian Crisis: A Case-Control Study

Z. Khrait[1], R. AL-Nasser[2]

[1]Fakih Fertility Center, IVF, Dubai, United Arab Emirates ;
[2]British-Syrian IVF & Fetal Medicine Center, Fertility, Damascus, Syria

Study question: To explore the differences in semen analysis parameters among Syrian males before and during the Syrian crisis to identify the impact of war on male fertility.
Summary answer: Semen samples obtained during the Syrian war contained semen in significantly higher quantities but of lower quality. Sperm morphology is subject to aberrations.
What is known already: Several studies conducted on veterans and civilians exposed to war-related injurious trauma showed that war operations and trauma had no impact on male infertility in terms of fathering a child after exposure to war incidents.
Study design, size, duration: Control samples were collected in 2009-2010 for 847 participants (before the war), and case samples were collected in 2014-2015 for 1108 participants (during the war). The semen analysis parameters of each case were obtained electronically from hospital records. Both verbal consent and ethical approval were secured. Data analyses were conducted with SPSS version 23.0 with 95% confidence intervals.
Participants/materials, setting, methods: A total of 1108 male cases from the war in Syria (2014-2015) were included in the study and were compared with 847 controls (2009-2010). Exclusion criteria included a BMI of greater than 30, age older than 55 years, confirmed diagnosis of systematic disease, extensive exposure to radiation and chemicals not related to wartime events (such as exposures among radiologists and chemists), and administration of medications with a known impact on fertility.
Main results and the role of chance: The mean semen volume was significantly higher in cases (3.18 ± 1.68 ml vs 3.45 ± 1.75 ml) than in controls (p <.001). In contrast, the means of all other parameters (density; motility categories A, B, and C; and percentage of normal sperm) were significantly lower among cases than among controls (all P-values <.001).
Conclusion: Semen samples obtained during the Syrian war contained semen in significantly higher quantities but of lower quality. Sperm morphology is subject to aberrations that highlight the risk of fertility problems. Motility and normal sperm percentage are the most affected parameters.
Limitations, reasons for caution: While the findings of our study are promising, the extent to which psychological stress affects spermatogenesis remains unclear. A large randomized controlled trial is recommended to fully elucidate the specific confounding factors implicated in spermatogenesis and to understand the effect of stress, particularly war, on the deterioration of sperm quality.
Wider implications of the findings: It is recommended that fertility health should an area of focus for those exposed to wartime events.
Trial registration number: not applicable

P-102 Microfluidic sperm selection for couples with a history of aneuploid embryos

A. Parrella[1], D. Tavares[1], M.S. Wang[1], M. Haddad[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Does selecting the highest progressively motile spermatozoa with optimal genomic integrity enhance the likelihood of generating euploid embryos and achieving higher pregnancy rates?
Summary answer: Microfluidic sperm selection (MFSS) identified spermatozoa with the highest chromatin integrity, leading to higher implantation and delivery rates.
What is known already: Genomic impairment of the male gamete can hinder embryo cleavage and implantation. Dysfunction of the male genital tract increases both single-strand (ss) and double-strand (ds) DNA nicks and breaks that can

inhibit the developmental competence of embryos. In particular, ds DNA breaks present in the spermatozoa of fertile donors, at a proportion as high as 40%, may contribute to embryo aneuploidy with consequent implantation impairment.
Study design, size, duration: From October 2016 through January 2020, 26 consenting couples underwent a new ICSI cycle in which spermatozoa were selected by MFSS. Most couples had a history of high sperm chromatin fragmentation (SCF) in their ejaculate, a high proportion of aneuploidy embryos, or recurring implantation failure after ICSI.
Participants/materials, setting, methods: SCF was measured by TUNEL on raw semen specimens as well as after density gradient centrifugation (DGC) and MFSS. ICSI was carried out with spermatozoa selected by the two different methods, and resulting embryos underwent preimplantation genetic testing for aneuploidy (PGT-A). Fertilization and clinical pregnancy outcomes, following replacement of thawed euploid blastocysts, were recorded and compared between the two sperm selection methods.
Main results and the role of chance: A total of 26 men (39±7 years) had a mean sperm concentration of 43.3±41.6x10^6/mL, 34.6±17 motility, and 2.4±1% morphology. After DGC and MFSS, the sperm concentration was 4.1±5 and 3.4±4x10^6/mL, with 70±32% and 95±15% motility, respectively (*P*<0.0001). Average SCF decreased from 22% in raw samples to 18% following DGC, and became 2.8% after MFSS processing (*P*<0.0001).

A total of 14 men underwent 29 ICSI cycles with their female partners (37.3±5 years) with DGC-selected spermatozoa, achieving a 56.7% fertilization rate (177/312), resulting in 63.2% (55/87) morphologically good-quality embryos; 18.4% were euploid at time of transfer in 9 couples, resulting in a 21.4% (3/14) CPR and 33% pregnancy loss (1/3). Subsequently, they underwent ICSI with MFSS, achieving a 74% fertilization rate (137/186; *P<0.001*), with 61% (51/84) good-quality embryos of which 45.2% (38/84) were euploidy *(P<0.001*). A total of 10 couples received a replacement, achieving a 75% implantation rate (9/12; *P*<0.001), resulting in a 90% CPR/cycle (9/10); most delivered (*P*<0.001) or are ongoing.

Next, we treated solely with MFSS 12 couples with a history of all aneuploid embryos at an outside center. They underwent ICSI at our center, achieving an 85% fertilization rate, a 60% (9/15) implantation rate, and the same CPR.
Limitations, reasons for caution: This study represents a preliminary experiment on a small number of subjects. While the oocyte contribution to aneuploidy cannot be discounted, MFSS was able to yield the highest progressively motile spermatozoa with optimal genomic integrity capable of enhancing the chances of generating euploid embryos.
Wider implications of the findings: The occasional presence of ds-DNA in the male gamete has been considered responsible for increasing chromosomal structure abnormality. MFSS of highly motile and genetically competent male gametes may enhance the chances of obtaining a euploid conceptus for transfer.
Trial registration number: not applicable

P-103 Comparison the ICSI outcomes of three protocols including fresh and frozen-thawed testicular sperm and donor semen (AID) for non-obstructive azoospermia (NOA) patients

L. Zhang[1]

[1]*li.zhang, Department of Obstetrics and Gynecology, Beijing, China*

Study question: Are there different outcomes of intracytoplasmic sperm injection (ICSI) in NOA patients with fresh and frozen-thawed testicular sperm versus AID sperm?
Summary answer: Comparing similar outcomes with fresh and frozen-thawed testicular sperm, NOA patients showed best ICSI outcome with AID sperm.
What is known already: Approximately 60% of azoospermia cases are due to NOA caused by testicular dysfunction.NOA patients can become fathers through micro-TESE which is associated with a high sperm retrieval rate (SRR) of 30-75%. So they have two treatments either synchronous oocyte retrievals for their partners or testicular sperm cryopreserved when they could gain sperm by surgery.
Study design, size, duration: Comparing the outcomes of NOA patients both undergoing ICSI with fresh and frozen-thawed testicular sperm versus NOA patients undergoing ICSI with AID sperm was conducted to achieve this goal after excluding infertility caused by female factors.
Participants/materials, setting, methods: We analyzed the outcomes of 474 NOA patients undergoing 413 ICSI cycles with fresh testicular sperm, 198 ICSI cycles with frozen-thawed testicular sperm and 170 azoospermic patients with donor sperm undergoing 197 ICSI cycles between January 2015 and March 2019. The parameters were fertilization rate (FR), 2PN cleavage rate (2PNCR), blastocyst formation rate (BFR), implantation rate (IR), cumulative pregnancy rate (CPR), cumulative live-birth rate (CLBR), miscarriage rate (MR) and no embryo available cycle rate (NEACR).
Main results and the role of chance: Comparing fresh and frozen-thawed testicular sperm, NOA patients using AID sperm showed the better outcome after ICSI-ET (for FR, BFR, IR, CPR, CLBR and NEACR, all p values were 0.000). NOA patients had the similar ICSI outcomes with fresh and frozen-thawed testicular sperm and this results were not effected by male female age, female BMI, male age, male BMI, male FSH, male LH and male T after adjusting confounding factors by linear and logistic regression analyse.
Limitations, reasons for caution: The small number of transfer cycles for NOA patients (due to the exclusion criterias) reduced the precision of our estimates. However, our results provide a valuable indication of the ICSI outcome for NOA patients using fresh or frozen-thawed testicular sperm.
Wider implications of the findings: Our results provide a valuable information about similar ICSI with fresh or frozen-thawed testicular sperm for counselling of NOA patients. They maybe choice AID sperm if they want a better ICSI outcome.
Trial registration number: 81521002

P-104 Does sperm DNA fragmentation influence blastocyst ploidy?

D. Mavrelos[1], **B. Sowry**[2], **B. Jones**[3], **G. Morris**[1], **S. Lewis**[4], **J. Ben Nagi**[5]

[1]*University College London Hospital, Reproductive Medicine Unit, London, United Kingdom ;*
[2]*Centre for Reprodutive and Genetic Health, Embryology, London, United Kingdom ;*
[3]*Imperial College London, Reproductive Medicine, London, United Kingdom ;*
[4]*Queen's University Belfast, Reproductive Medicine, Belfast, United Kingdom ;*
[5]*Centre for Reprodutive and Genetic Health, Reproductive Medicine, London, United Kingdom*

	Fresh testicular sperm	Frozen testicular sperm	AID sperm	P-value (fresh vs. frozen)	P-value (fresh vs. AID)	P-value (frozen vs. ADI)
FR% (n)	44.8 (2172/4844)	49.22 (1036/2105)	70.11 (1581/2255)	0.001	p<0.001	p<0.001
BFR% (n)	26.31 (241/916)	22.40 (99/442)	33.79 (197/583)	0.119	0.002	p<0.001
IR% (n)	29.61 (215/726)	26.18 (83/317)	34.92 (161/461)	0.259	0.055	0.010
CPR% (n)	42.51 (176/414)	35.86 (71/198)	60.91 (120/197)	0.117	p<0.001	p<0.001
CLBR& (n)	28.99 (120/414)	25.25 (50/198)	50.76 (100/197)	0.795	p<0.001	p<0.001

Study question: Is high sperm DNA fragmentation associated with reduced blastocyst euploid rate?
Summary answer: We show a significantly lower euploid blastocyst rate in couples with high sperm DNA fragmentation.
What is known already: Sperm DNA fragmentation has been associated with clinical pregnancy miscarriage. A putative mechanism is karyotypic abnormalities in the blastocysts resulting from conception with sperm with high DNA fragmentation.
Study design, size, duration: We included 174 couples undergoing treatment during the study period. Data for each ART cycle was held on IDEAS version 6 Mellowood Medical and analysed using SPSS version 24.0.

Female and male partners age was grouped into age ranges <35; 35 – 37; 38 – 39; 40 – 42; >42 years for analysis.
Participants/materials, setting, methods: We included 174 couples undergoing treatment during the study period. Data for each ART cycle was held on IDEAS version 6 Mellowood Medical and analysed using SPSS version 24.0.

Female and male partners age was grouped into age ranges <35; 35 – 37; 38 – 39; 40 – 42; >42 years for analysis.
Main results and the role of chance: Median maternal age was 39 years (IQR 37 – 41) and paternal age 40 years (36 – 44). During the study period 797 blastocysts were biopsied and 246 (31%) were reported euploid. Euploidy rate by maternal age was <35; 78/155 (50%), 35 – 37; 72/171 (42%), 38 – 40; 65/173 (27%), 41- 42 27/150 (15%) and >42; 4/56 (7%). Couples with a female partner under 35 and abnormal sperm DFI had a significant lower euploidy rate compared to couples with normal DFI (31/76, 41% vs. 47/79, 59%), p=0.0198. There was no significant difference in older age groups, 35 – 37 (45/119, 38% vs. 27/52, 52%) p=0.085, 38 – 40 (38/130, 29% vs. 27/108, 25%) p=0.465, 41 – 42 (18/96, 19% vs. 9/72, 11%) p= 0.159 and >42 (4/43, 9% vs. 0/9, 0%), p=0.17 .
Limitations, reasons for caution: This is a small retrospective study with important clinical implications but prospective studies are needed for confirmation.
Wider implications of the findings: Abnormal sperm DNA fragmentation is associated with less euploid blastocysts in couples with a female partner under 35 years of age.
Trial registration number: NA

P-105 Impact of male infertility fellowship training on the vasectomy reversal learning curve

G. Wood[1], E. De Paula Miranda[2], F. Tenorio Lima Neto[3]

[1]Urologist, Urology, Sao Paulo, Brazil ;
[2]Universidade Federal do Ceará, Urology, Fortaleza-CE, Brazil ;
[3]Instituto de Medicina Integral Professor Fernando Figueira, Urology, Recife-PE, Brazil

Study question: How many vasectomy reversal procedures are needed for a trained microsurgeon to achieve good patency results and reduce operative time?
Summary answer: Vasectomy reversal is a challenging procedure, and at least 55 procedures are needed to achieve a good surgical time.
What is known already: Male infertility microsurgery (MIM) is an area in intense development, requiring intensive surgeon training and its results are highly dependent on surgeon's skills and domain of microsurgical techniques. Vasectomy reversal (VR) is the most cost-effective option for couples desiring offspring after vasectomy, and is one of the most difficult microsurgical procedures. We report the learning curve results for VR performed by a single surgeon after his MIM fellowship training.
Study design, size, duration: In a retrospective cohort fashion, we reviewed the charts of all patients who underwent VR from June 2016 to August 2019 by a single surgeon who finished a MIM fellowship training in May 2016. All procedures were performed using an operating microscope. A multilayer technique was used for vasovasostomies (VV), and the LIVE technique for vasoepididymostomies.
Participants/materials, setting, methods: At the end of the study duration, 69 patients were included. They were divided into tertiles by dates. We assessed and compared the following variables: surgical time, complications, postoperative semen analysis parameters, patency, and pregnancy rates.
Main results and the role of chance: 26 patients were included in each tertile. There were no differences between tertiles regarding baseline characteristics. The proportion of men who underwent bilateral VV decreased from the first to the third tertile (88% to 62%, p = 0.08). Although this difference was not statistically significant, we believe that it might have impacted the results of the third tertile. The surgical time progressively reduced from the first through the third tertile, and the trend curve reached a plateau after 55 cases (203 to 156 minutes, p < 0.01). Patency rates were high since the first tertile (85%, 90% and 93%, from the first to the third tertile, p = 0.75). There were no statistical differences regarding the patency rates among the groups, but they seemed to improve through the tertiles. Likewise, the post-operative total sperm count had a clinically, but not statistically, significant improvement through the tertiles (88%, 93% and 100%, p = 0.78). Meanwhile, the post-operative total motile sperm count was similar among the groups (33, 22 and 26 million/mL, consecutively). The overall pregnancy rate for patients with more than 12 months of follow up was 42%.
Limitations, reasons for caution: The retrospective nature of the results presented here should be taken into account before the generalization of our conclusions. Additionally, the surgeon was submitted to intense microsurgery training before the beginning of this study, which may not be the case for all urologists interested in the infertility field.
Wider implications of the findings: Vasectomy reversal is a challenging procedure, and at least 55 procedures are needed to achieve a good surgical time. MIM fellowship training provides microsurgical skills that help to accelerate the procedure learning curve.
Trial registration number: Not applicable

P-106 Contribution of sperm genomic and extragenomic markers as a critical determinant of embryonic development and embryo viability in spontaneous and assisted conceptions

V. Dhawan[1], D. Deka[2], N. Malhotra[2], V. Dadhwal[2], N. Singh[2], R. Dada[1]

[1]All India Institute Of Medical Sciences, Anatomy, New Delhi, India ;
[2]All India Institute Of Medical Sciences, Obstetrics & Gynaecology, New Delhi, India

Study question: Does the transmission of sperm genomic and extragenomic cargo hold any relevance in early embryonic development and embryo viability in successful pregnancy outcomes?
Summary answer: The dynamic intertwined role of sperm DNA and RNA elements delivered at fertilization have postfertilization functions and navigate early embryonic development in successful pregnancy outcomes.
What is known already: The suite of complex repertoire of sperm RNA delivered to the oocyte at fertilisation have the potential to navigate the early embryonic development. The epigenetically marked sperm genome successfully orchestrates in synchrony with the extragenomic cargo. Sperm with disturbed genomic integrity and dysregulated levels of sperm RNA may be responsible for a successful fertilisation but may affect the pregnancy outcomes and even affect the health of the offspring.
Study design, size, duration: A case control study of male partners of couples experiencing RPL (n=75), recurrent implantation failure (n=75) and 30 controls at AIIMS, New Delhi, India. Study duration was 2 years.
Participants/materials, setting, methods: A case control study of male partners of couples experiencing RPL (n=75), recurrent implantation failure (n=75) and 30 controls at AIIMS, New Delhi, India.
Main results and the role of chance: The transcript levels of *SOX3, WNT5A, RPS6, RBM9, RPL10A* showed significant difference beyween RPL patients and controls. While the levels of *FOXG1, RPS6, RBM9, RPL10A, RPS17* and *TOMM7* showed significant difference between RIF patients and controls. The mean ROS and DFI was seen to be significantly higher (in both RPL and RIF patients as compared to controls in both RPL and RIF patients (p <0.001). The odds of occurrence of RPL and RIF was 12.41 and 13.68 times higher, whose ROS> 28 % [(OR 12.41, 95% CI: (6.28-22.29) and OR 13.68 (6.52-28.71)] (p<0.001). While the odds of occurrence of RPL and RIF 12.68 and 18.87 times higher, whose DFI>30 (OR 12.68, 95% CI: (6.53-23.55) and OR 18.87 (6.52-28.71), (p<0.001). The mean sperm telomere length was seen to be lower in RPL (p=0.054) and RIF (p=0.0294) patients with respect to controls.
Limitations, reasons for caution: A potential limitation of this study is the smaller sample size recruited for the current study. Also the contribution of maternal factors and the possible role of other environmental factors which may affect implantation and early embryogenesis cannot be ignored.

Wider implications of the findings: The complex interplay of the various transcripts have seen to have significant effects in pregnancy outcomes and health trajectory of the future progeny. Any perturbation in this has the potential to affect the early embryonic development and embryo viability. This may further alter sperm methylation pattern adversly affecting sperm epigenome.
Trial registration number: Not Applicable

P-107 Epigenetic Assessment by RNA Sequencing of Non-Obstructive Azoospermic Men to Predict Successful Testicular Sperm Retrieval

K. Hancock[1], A. Parrella[1], S. Cheung[1], B. Barker[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Can epigenetic profiling of key genes provide information on the germinal epithelium function of men with non-obstructive azoospermia (NOA) and the likelihood of retrieving spermatozoa?
Summary answer: By sequencing transcripts in NOA men, we identified genes related to maturational arrest in men with different sperm retrieval outcomes with testicular sperm extraction (TESE).
What is known already: Azoospermia accounts for about 15% of male infertility cases, and while it is rarely caused by pre-testicular factors, the most common forms are testicular and post-testicular. The more challenging type is secretory azoospermia, in which scattered persistent germinal epithelium strive to support the germline meiotic process, failing to yield spermatozoa via testicular biopsy in almost 50% of cases. To judge the reproductive potential of these individuals, we assessed the gene expression profile to predict the ability to identify injectable gametes.
Study design, size, duration: Over a 3-month period, we executed a prospective study on 3 consenting men who yielded no sperm after extensive semen analysis. Differential expression was carried out on the ejaculate and compared to a fertile donor. Subsequently, they underwent TESE, and gene expression analysis was carried out both in comparison to a man with obstructive azoospermia (OA), as well as their ejaculated specimen, in relation to whether spermatozoa were retrieved with TESE.
Participants/materials, setting, methods: Three men presented with no spermatozoa in their ejaculates after an extensive sperm search and were diagnosed as NOA. Subsequently, they underwent TESE. RNA expression was performed on the leftover ejaculate specimen and on the testicular specimens from these individuals. Purified RNA was sequenced by Illumina HiSeq at 2x150bp per lane with ~58M reads/sample. A log2fold change of >1 and $P<0.001$ were considered significant.
Main results and the role of chance: Three consenting men (36.3±5yrs) were included in this study. All underwent extensive semen analysis of their ejaculate, and no spermatozoa were found. These men were diagnosed with NOA following urological evaluation. Quantitative analysis of RNA extracted from these ejaculated samples averaged a concentration of 2.3±1 ng/ul and an RNA integrity of 7.0±1. Compared with the ejaculated donor, we found ~3000 genes differentially expressed for each man, with 1379 in common, most associated with meiotic arrest (TEX11, DMC1, MEOB, MEI1, STX2).

These men underwent TESE and a gene expression analysis in comparison with an OA control, ~3000 genes were identified, with 702 in common, including TSPYL1 and TSPYL2, associated with spermatogenesis.

Spermatozoa were successfully identified in the TESE specimen of 2 men (38.5±6yrs) at a 0.08±0.1x10^3/ml concentration. RNA concentration was 5.57±1 ng/ul with 7.0±1 integrity. Genes associated with cell proliferation (*MAPRE1*, *RAN*), apoptosis (*ARL6IP1*), embryo development (*IL6ST*), and spermatogenesis (*AKAP11*) were underexpressed in the ejaculate compared to testicular biopsy ($P<0.001$).

In a 35-year-old man with no spermatozoa in testicular biopsy, RNA-sequencing revealed several genes that were uniquely overexpressed in the ejaculate compared to the testicular. These were associated with spermatogenesis(*DAZAP2*, *SYNJ2BP*), cell cycle arrest(*CAB39*), DNA replication(*NFIB*), and cilia formation(*IFT57*) ($P<0.001$).
Limitations, reasons for caution: This is a preliminary analysis, which needs to be corroborated by further observations. The source of the RNA is inconsistent because in the case of spermatozoa absence, only cells from the male genital tract and from the compromised geminal epithelium were available.
Wider implications of the findings: Our epigenetic analysis demonstrated that key genes related to spermatogenesis were unbalanced, suggesting a maturational arrest as the cause for the azoospermia. The identification of specific genes in men with failed TESE may provide valuable information on the ability to successfully predict the likelihood of retrieving spermatozoa with TESE.
Trial registration number: not applicable

P-108 Can Cytomegalovirus (CMV) infection affect male reproductive function? Results of a retrospective single-center analysis

A. Natali[1], G. Cito[1], R. Picone[2], R. Fucci[2], E. Micelli[2], A. Cocci[1], M. Di Mauro[3], C. Giachini[2], F. Rizzello[2], A. Minervini[1], M. Carini[1], M.E. Coccia[2]

[1]Careggi Hospital - University of Florence, Dept. of Urology, Florence, Italy ; [2]Careggi Hospital - University of Florence, ARTs Center, Florence, Italy ; [3]University of Catania, Dept. of Urology, Catania, Italy

Study question: To investigate the impact of chronic CMV infection on semen parameters in men with couple infertility and the influence on the reproductive outcomes of CMV-seronegative women suffering from tubal factor
Summary answer: CMV did not seem to play a key role in male reproductive function, as well as in influencing sperm fertility potential in assisted reproductive outcomes.
What is known already: Cytomegalovirus (CMV) is mainly investigated for the consequences of prenatally transmission from mother to fetus, that can lead to severe disturbances of development. However, the impact of infection on the male reproductive health has been received little consideration, despite a potential negative effect of the virus on the spermatogenesis.
Study design, size, duration: From February 2016 to January 2019, all the couples referring to our ARTs Centre for infertility due to female tubal factor, were retrospectively reviewed. All the men were divided into two groups: Group 1 included CMV IgG-seropositive men, Group 2 comprised CMV IgG-seronegative patients.
Participants/materials, setting, methods: On the day of fresh IVF/ICSI treatment, we collected data of the seminal characteristics, as follows: volume, pH, total sperm count/ml, total sperm concentration, viability, progressive motility (PR), non-progressive motility (NP), immobility, total motility and normal morphology. Two-pronuclear (2PN) fertilization rate (FR), 1-2-3PN FR, cleavage rate (CR), miscarriage rate (MR), pregnancy rate (PR) and live birth rate (LBR) were collected.
Main results and the role of chance: 222 men were included: 115 (51.8%) in the Group 1, 107 (48.2%) in the Group 2. There was reported a low trend towards higher sperm concentration/ml, total sperm count and viability in CMV IgG-seronegative males, compared to CMV IgG-seropositive, but no statistically differences were found among groups. Semen volume, pH, motility and normal sperm morphology were similar between the two groups. Considering the subgroup of men, partners of CMV IgG-seronegative females, 65 couples (29.2%) were selected. Overall, median 2PN FR was 67%, total FR 83%, CR 100%, PR/cycle 26.2%, MR 10.8%, LBR/cycle 15.4%. No significant differences were found regarding the reproductive and pregnancy outcomes between CMV IgG-seropositive men and those seronegative.
Limitations, reasons for caution: We did not test the presence of CMV DNA in the seminal samples, due to the time and cost of performing polymerase chain reaction (PCR) and cell culture, compared to the more rapid and inexpensive detection of CMV IgG and IgM assays.
Wider implications of the findings: The viral infection did not seem to play a key role in male reproductive function and in influencing sperm fertility potential in the assisted reproduction. CMV serology screening could be crucial to identify primary acute infection by detecting IgM seropositivity, in order to prevent virus transmission by sperm sample.
Trial registration number: not applicable

P-109 Impact of male body mass index on Invitrofertilisation outcome-Live birth rate.

R. Mushtaq[1], C. Yearwood[2], Y. Khalaf[2], T. El-Toukhy[2]

[1]CARE Fertility United Kingdom, Clinical, Bedford, United Kingdom ;
[2]Guy's and St Thomas foundation trust hospital, Assisted conception unit, London, United Kingdom

Study question: Does male body mass index effect outcome of assisted reproductive technology?
Summary answer: Male body mass index does not influence Invitrofertilisation live birth rate after adjusting for important confounding factors.
What is known already: Male factor infertility could be contributory in up to 50% of cases seen in assisted reproduction clinics. Obesity is becoming pandemic and is contributory factor in overall fall noted in semen parameters worldwide. There are many studies on impact of female BMI on outcomes of IVF. There is conflicting evidence regarding the effect of raised paternal BMI on outcome of assisted reproductive technology. Most of the studies are small and retrospective.
Study design, size, duration: It is a prospective observational study, including 438 couples having IVF or ICSI treatment at Guy's and St Thomas' hospital, London between July 2015 and June 2016.
Participants/materials, setting, methods: Couples undergoing first or second IVF cycle between July 2015 to June 2016 where recruited before starting the IVF cycle. Both male and female BMI were measured and recorded at the start of treatment. All IVF/ICSI cycle characteristics were recorded and all pregnancies followed up till delivery
Main results and the role of chance: Of the 438 couples included in the study, 165 (38%) male partners had a normal BMI (18.4-24.9) and 273 (62%) had a raised BMI (25-41.9). There was no significant difference between the two groups in sperm parameters. The clinical pregnancy and live birth rates were not significantly different between the two groups (33% vs 35% and 25% vs 31%, respectively, P=0.19). After adjusting for important confounders, including female partner age, female BMI, duration of infertility, pre-treatment AMH level, total dose of gonadotrophins used during ovarian stimulation, number of oocytes collected, method of oocyte fertilisation (IVF vs ICSI) and number of embryos transferred, the likelihood of a live birth outcome was not significantly different in the raised male BMI group compared to normal male BMI group (OR 1.22, 95% CI 0.69-2.14, P=0.49). There was no significant difference in the live birth rate per cycle between overweight (BMI 25-29.9, n=193) and obese (BMI 30-41.9, n=80) male partners (28% vs 38%, P=0.1).
Limitations, reasons for caution: These results are from prospective but small study with live birth rate as the end point .
Wider implications of the findings: Larger proportion of men had high BMI in our study. This could be reflective of rising obesity. While our study has not shown a significant impact of male BMI on live birth rate in IVF cycles, the health implications of high BMI in population should be considered. .
Trial registration number: not applicable

POSTER VIEWING SESSION
EMBRYOLOGY

P-110 Irregular cleavage in early embryogenesis does not reduce the euploidy after reaching the blastocyst

S. Watanabe[1], K. Yoshikai[1], M. Tomida[1], S. Suzuki[1], Y. Matsuda[1], T. Kato[2], E. Nakano[1], H. Kurahashi[2], T. Sawada[1]

[1]Sawada Women's Clinic, ART Lab., Nagoya, Japan ;
[2]Institute for Comprehensive Medical Science- Fujita Health University, Division of Molecular Genetics., Toyoake, Japan

Study question: Does euploid rate decrease when the irregular first or second cleavage embryos reach the blastocyst?
Summary answer: The euploidy of the irregular first or second cleavage blastocysts was equivalent to the normal cleavage blastocysts.
What is known already: There are some reports that aneuploidy is high when the irregular cleavage embryos develop into blastocysts but there are reports that the rate of transfer pregnancy and miscarriage of irregular cleavage blastocysts is equivalent to that of the normal cleavage blastocysts.
Study design, size, duration: Retrospective analysis was conducted on 96 discarded blastocysts with consent from patients out of embryos collected and cultured in our clinic from 2013 to 2018. All of these embryos were time-lapse monitored by EmbryoScope (Vitrolife).
Participants/materials, setting, methods: The subject embryos were biopsied with TE and NGS chromosome analysis was performed. These embryos were classified by time-lapse videos, those with 2 cells in the first cleavage and 4 cells in the second cleavage are the good cleavage groups, those with 3 or more cells in the first cleavage, or those with 5 cells or more in the second cleavage are the irregular cleavage groups.
Main results and the role of chance: As a result of image analysis by time-lapse monitoring, there were 53 good cleavage groups and 43 irregular cleavage groups. The proportions of euploid embryos, aneuploid embryos and mosaic embryos were 43.4% (23), 45.3% (24) and 11.3% (6) in the good cleavage group, 41.9% (18), 46.5% (20), and 11.6% (5) in the irregular cleavage group, there was no significant difference in euploid rate between the two groups (The odds ratio of the euploid rate of the irregular cleavage group to the good cleavage group was 0.94).
Limitations, reasons for caution: Because PGT-A is not approved in Japan, this study was conducted using only discarded embryos that were not used for transfer with the consent of the patient.
Wider implications of the findings: This study showed that Irregular cleavage in early embryogenesis did not reduce the euploid rate after reaching the blastocyst. Irregular cleavage embryos that have reached the blastocyst need not be excluded from transfer.
Trial registration number: not applicable

P-111 MicroRNAs secreted by human embryos are potential biomarkers for clinical outcomes of assisted reproductive techniques

Z. Li[1], F. Fang[2], X. Chengliang[1]

[1]Huazhong University of Science and Technology, Institute of Reproductive Health, WuHan, China ;
[2]Huazhong University of Science and Technology, Department of Obstetrics and Gynecology- Union Hospital- Tongji Medical College, WuHan, China

Study question: Investigate whether the miRNAs secreted by human embryos in culture medium can be used as embryonic biomarkers.
Summary answer: Our results indicate that miRNAs in human embryo culture media may serve as novel and invasive biomarkers for embryo selection during IVF/ICSI-ET cycles.
What is known already: MicroRNAs (miRNAs) are important regulators of many biological functions, including embryo implantation and development. Recently, it is reported that miRNAs in biofluids are predictive for physiological and pathological processes.
Study design, size, duration: The culture media were prospectively collected from embryos of patients who underwent routine *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) at reproductive medicine center with informed consent. And
Participants/materials, setting, methods: A high-throughput miRNA sequencing method was applied to detect the miRNAs profiles in culture media of embryos with different reproductive outcomes (15 samples were mixed for each group). Furthermore, qRT-PCR and droplet digital PCR (ddPCR) to verify the target miRNAs at single sample level (100 cleavage samples in total, 50 with successful pregnancy and 50 with failed pregnancy). Receiver operating characteristic (ROC) analyses were performed for differentially expressed miRNAs.
Main results and the role of chance: The miRNA sequencing showed that embryos with successful pregnancy secreted different miRNA profiles into the culture media, compared with embryos with failed pregnancy. These differentially expressed miRNAs were predicted to be involved in multiple biological processes, cellular components and molecular functions. And 18 miRNAs were selected for validation by qRT-PCR in mixed samples and significantly different expression of these 10 miRNAs between two groups were identified. The ddPCR results revealed that hsa-miR-26b-5p, hsa-miR-451a and hsa-miR-21-5p could be stably detected in the culture medium of one single embryo at cleavage stage. After statistical analysis, we found that the cleavage embryos with successful pregnancy presented decreased expression of hsa-miR-26b-5p and hsa-miR-21-5p in the culture media. Moreover, the Receiver Operating Characteristic

(ROC) curve analysis indicated that hsa-miR-26b-5p and hsa-miR-21-5p could serve as potential biomarkers for reproductive outcomes.
Limitations, reasons for caution: More clinical trials are needed to determine the sensitivity and specificity of miRNA biomarkers for embryo selection and more basic research are also necessary to develop better detection methods of miRNAs with low input.
Wider implications of the findings: Together, our findings highlight the important predictive potential of miRNAs secreted by human embryos in culture media, which is meaningful for noninvasive embryo selection during IVF cycles.
Trial registration number: none

P-112 An analysis of 2.1PN (2PN+1 micronuclei) formation.

N. Okuyama, MSc[1], N. Oka[1], N. Aono[1,2], T. Hashimoto[1], K. Kyono[1,2]

[1]Kyono ART Clinic Takanawa, Department of Gynecology, Tokyo, Japan ;
[2]Kyono ART Clinic Sendai, Department of Gynecology, Sendai, Japan

Study question: What factor causes 2.1 PN zygotes?
Summary answer: High-aged female patients (OR: 1.17, 95%; CI: 1.09-1.25; *P*<0.001) and frozen-thawed oocytes (OR: 3.85, 95%; CI: 1.79-8.26; *P*<0.001) were more likely to produce 2.1PN zygotes.
What is known already: We confirm fertilization when two pronuclei (PN) and second polar body are seen, and in some cases, 2PN + micronuclei (2.1PN) is recognized. It is reported that some 2.1PN zygotes may develop to blastocyst and lead to successful clinical pregnancy and live birth; however, there is a risk that 2.1PN zygotes have the possibility of triploid (Capalbo et al., 2017). A limited number of studies have reported on 2.1PN, but its mechanism is still unclear.
Study design, size, duration: This retrospective single-center study was performed from January 2015 to December 2018 including a total of 659 patients and 1161 cycles. A total of 7939 oocytes were inseminated either by conventional IVF (cIVF) or ICSI. Logistic regression analysis was conducted to investigate the cause of 2.1PN. Inseminated oocytes were cultured in time-lapse monitoring system. We defined a 2.1PN when two equal-sized PN, second polar body, and less than 1/3 the size of micronuclei were observed.
Participants/materials, setting, methods: We analyzed the associations between the number of PN and embryo development; background profiles such as female age, serum AMH, and BMI; ICSI or cIVF; ejaculated sperm or TESE sperm; fresh oocytes or vitrified-warmed oocytes; and with or without artificial oocyte activation (AOA). Good quality blastocyst rates were defined according to Gardner's criteria. Chi-square test and logistic regression analysis were conducted where appropriate.
Main results and the role of chance: Fertilization results were as follows: 26.7% (n=2122) for 0PN, 3.2% (n=255) for 1PN, 62.1% (n=4928) for 2PN, 1.0% (n=83) for 2.1PN, 4.1% (n=328) for 3PN, and 2.8% (n=223) for >3PN. There were statistically significant differences in the blastocyst formation rates and good quality blastocyst rates between 2PN, 2.1PN and 3PN embryos, (49.6% vs 30.1% vs 14.3%, *P*<0.001) (34.0% vs 20.5% vs 7.0%, *P*<0.001). High-aged female patients (OR: 1.17, 95%; CI: 1.09-1.25; *P*<0.001) and vitrified-warmed oocytes (OR: 3.85, 95%; CI: 1.79-8.26; *P*<0.001) were more likely to obtain 2.1PN zygotes. No association was found between 2.1PN formation and serum AMH (OR: 0.96, 95%; CI: 0.86-1.06; *P*=0.448), BMI (OR: 0.96, 95%; CI: 0.89-1.03; *P*=0.295), ICSI or cIVF (OR: 1.33, 95%; CI: 0.61-2.11; *P*=0.702), ejaculated or TESE sperm (OR: 1.40, 95%; CI: 0.65-3.00; *P*=0.392) and with or without AOA (OR: 1.18, 95%; CI: 0.28-5.09; *P*=0.821).
Limitations, reasons for caution: PGT-A was not analyzed due to the regulation of the Japan Society of Obstetrics and Gynecology.
Wider implications of the findings: This study indicated that 2.1PN may be derived from oocyte factors such as advanced female age and oocyte cryopreservation. 30.1% of 2.1PN zygotes developed to blastocyst; however, 2.1PN zygotes may be triploid (Capalbo et al., 2017). Hence, a careful choice for embryo transplantation is needed.
Trial registration number: none

P-113 Fertilization by direct pressing of zona pellucida-bound human sperm onto the oocyte membrane

S. Hatakeyama[1,2], Y. Araki[2], Y. Araki[2], S. Ohgi[1], A. Yanaihara[1]

[1]Yanaihara Women's Clinic, Obstetrics and Gynecology, Kamakura, Japan ;
[2]Gunma Paz University, Graduate School of Health Sciences, Takasaki, Japan

Study question: Can fertilization be obtained by pressing single sperm onto an unfertilized oocyte after in vitro fertilization (IVF) without breaking the oocyte membrane?
Summary answer: Fertilization was obtained by pressing zona pellucida (ZP)-bound sperm directly onto the oocyte membrane, a procedure that we designated as assisted sperm fusion insemination (ASFI).
What is known already: Rescue intracytoplasmic sperm injection (ICSI), which is ICSI of unfertilized oocytes after conventional IVF, has been reported to prevent total fertilization failure. However, recent studies have shown that the outcome of rescue ICSI is unsatisfactory, resulting in a poor fertilization rate and a high degeneration rate. A micromanipulation technique that permits fertilization without breaking the oocyte membrane would be expected to reduce further the degeneration rate after rescue ICSI.
Study design, size, duration: This study of the ASFI method was performed between January 2019 and December 2019 on 49 subjects. The results of ASFI were compared with those obtained in a previous trial of rescue ICSI that was performed between July 2018 and December 2018. The present study was approved by the local ethics committee.
Participants/materials, setting, methods: The acrosome reaction (AR) rate of 49 motile sperm collected from the ZP was assessed. Sixty-nine motile sperm bound to the ZP were aspirated directly using an injection pipette. These motile sperm were pressed onto the membrane of 69 unfertilized oocytes at 6 hours after IVF; pressing was performed with the injection pipette for 30 seconds.
Main results and the role of chance: The AR rate of sperm collected from the ZP was significantly higher than that of control sperm (98.0% vs. 28.6%, p <0.01). The mean and standard deviation of ages of the women in ASFI and rescue ICSI were 36.1 ± 3.9 and 37.7 ± 4.3 years, a difference that was not significant (p >0.05). All sperm collected from the ZP adhered to the oocyte membrane after ASFI. Time-lapse cinematography showed that sperm pressed onto the oocyte membrane remained there for approximately 30 minutes before being incorporated into the oocyte membrane. There was no significant difference in fertilization rate (69.6% vs. 70.0%), degeneration rate (0% vs. 4%), number of good-quality embryos at day 3 (58.3% vs. 51.4%), or blastocyst formation rate (58.5% vs. 42.4%) between the ASFI and rescue ICSI groups, respectively.
Limitations, reasons for caution: The major limitation of this study was the use of sperm bound to the ZP. For more general application, a method of artificially inducing AR will need to be established. Moreover, our sample size was relatively small; further studies with a larger number of patients will be needed.
Wider implications of the findings: ASFI is expected to improve the survival rate of oocytes and to increase the number of the embryos available for implantation. Additionally, ASFI may contribute to the elucidation of the mechanism of fertilization, given that the technique permits observation of the process of fusion between the oocyte and sperm.
Trial registration number: not applicable.

P-114 Non-invasive prediction of the blastocyst formation by morphokinetics discriminant analysis

A. Iaconelli Jr.[1], A. Setti[2,3], D. Braga[2,3], L. Vingris[4], E. Borges Jr.[1,3]

[1]Fertility Medical Group, Clinical department, São Paulo- SP, Brazil ;
[2]Fertility Medical Group, Scientific research, São Paulo- SP, Brazil ;
[3]Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Assistida, Scientific research, São Paulo, Brazil ;
[4]Fertility Medical Group, IVF laboratory, São Paulo- SP, Brazil

Study question: Is the discriminant analysis of embryo morphokinetics able to predict the blastocyst formation chance on the 5th day of embryo development?
Summary answer: Early kinetic parameters may predict which embryo is able to develop into blastocysts and those failing to blastulate.
What is known already: At the early stage of embryonic development, the transcription of embryonic genes is quiescent, and development is conducted by maternal proteins and RNAs. Subsequently, embryonic genome

activation (EGA) occurs, followed by the developmental control switching to the nuclear genome. Studies have suggested that morphological grade is not accurate enough to predict the developmental potential. Most embryos reach the cleavage-stage, regulated by maternal factors, and EGA never occurs. Extended embryo culture and transfer at blastocyst stage is an alternative which allows the selection of embryos at more advanced stages, after EGA, increasing the implantation rate and minimizes the multiple pregnancies risk.

Study design, size, duration: Kinetic data were analyzed in 139 patients and 1219 zygotes cultured until day 5 in a time-lapse incubator system, between March/2019 and November/2019. Timing to specific events from the point of insemination was determined using time-lapse imaging. A stepwise discriminant function analysis determined which kinetic markers discriminate between embryos that reached the blastocyst stage or not. Moreover, cut-off points were established halfway between the averages that were significantly different between the blastocyst and non-blastocyst embryos.

Participants/materials, setting, methods: This study was performed in a private university–affiliated IVF center. The analyzed kinetic markers were: pronuclei appearance (tPNa), timing to pronuclei fading (tPNf), timing to two (t2), three (t3), four (t4), five (t5), six (t6), seven (t7), and eight cells (t8). Durations of the second (t3-t2) and third (t5-t3) cell cycles (cc2 and cc3, respectively), as well as timing to complete synchronous divisions s1 (t2-tPNf), s2 (t4-t3) and s3 (t8-t5) were calculated.

Main results and the role of chance: A total of 563 embryos reached the blastocyst stage (46.15%). The discriminant function correctly classified 76.1% of original cases, best predicting blastocyst formation (95.0%). The cross-validated classification showed that overall 75.9% were correctly classified. In this model, mean t2 (25.29 ± 3.19 vs. 27.26 ± 5.33), t7 (54.21 ± 8.40 vs. 57.34 ± 11.62), s2 (1.43 ± 2.69 vs. 3.24 ± 4.64) and s3 (8.48 ± 7.84 vs. 13.75 ± 10.05) were significantly different ($p<0.001$) between the blastocyst and the non-blastocyst group, respectively. Cut-off points were established halfway between those averages, at 26.27 for t2, 55.78 for t7, 2.34 for s2 and 11.12 for s3, for the prediction of blastocyst development.

Limitations, reasons for caution: Retrospective nature of this study and the small sample size may be a reason for caution.

Wider implications of the findings: The identification of markers of the blastocyst formation potential may lead to the benefits of the extended embryo culture without exposing the embryo to the deleterious effects of the in vitro culture for an extended period of time (i.e. epigenetic changes in trophectoderm cells leading to abnormal implantation and placentation).

Trial registration number: Not applicable

P-115 Intracytoplasmic sperm injection (ICSI) is not superior to conventional in vitro fertilisation (IVF) in non-male factor infertility: a systematic review and meta-analysis

A. Finet[1], E. Arbo[2], M. Colombani[3], B. Darne[4], V. Gallot[5], V. Grzegorczyk-Martin[1], S. Languille[4], T. Freour[6]

[1]Clinique Mathilde, Assisted Reproductive Technologies, Rouen, France ;
[2]Gedeon Richter France, Medical Affairs, Paris, France ;
[3]Clinique Bouchard-Elsan, Institut de Médecine de la Reproduction, Marseille, France ;
[4]Monitoring Force, Statistical Analysis, Maisons-Laffitte, France ;
[5]Hôpital Antoine Béclère- Assistance Publique – Hôpitaux de Paris, Service de Médecine de la Reproduction et Préservation de la Fertilité, Clamart, France ;
[6]CHU de Nantes, Service de biologie et médecine de la reproduction, Nantes, France

Study question: Does ICSI perform better than conventional IVF in non-male factor infertility in terms of live birth rate (LBR)?

Summary answer: ICSI is associated with lower LBR per cycle than conventional IVF in non-male factor infertility.

What is known already: Over recent decades, the use of ICSI has noticeably increased and is now significantly more frequently used than conventional IVF in most countries. As the prevalence of male infertility has not increased concomitantly, the relevance of ICSI in non-male factor infertility can be questioned, especially when its economic and/or psychological consequences are considered. Numerous studies comparing ICSI and conventional IVF in various cases of non-male factor infertility report heterogeneous results, thereby supporting the need for a meta-analysis.

Study design, size, duration: Systematic review and meta-analysis of published research articles. Searches were conducted on MEDLINE, EMBASE, and the Cochrane Library from 2004 to June 2019 using medical subject headings and free text terms for 'ICSI', 'IVF', 'non male factor', 'unexplained infertility', 'fertilisation failure', 'pregnancy', 'live birth'.

Participants/materials, setting, methods: Studies preselected on title and abstract were assessed using adapted Newcastle-Ottawa Quality Assessment Scales. Only articles describing primary outcome of live birth were included. Risks of bias were assessed using ROBIN-I tools. Study selection, bias assessment and data extraction were performed by two independent reviewers according to Cochrane methods. Risk ratio (RR) IVF/ICSI and 95% confidence interval (CI) were estimated using random effect model. Pre-specified sensitivity analysis and meta-regression on mean female age were performed.

Main results and the role of chance: Thirty-nine full-text articles were preselected out of 1,615 references. Of these, 17 were included in the meta-analysis, corresponding to 395,414 assisted reproductive technology cycles (136,178 IVF and 259,236 ICSI cycles). IVF showed significantly higher LBR per cycle when compared to ICSI (RR=1.13; 95%CI=1.05-1.23). No statistical difference was found for clinical pregnancy rate per cycle (RR=1.09; 95%CI=0.93-1.29). Fertilization rate was significantly lower in IVF cycles (RR=0.80; 95%CI=0.71-0.89), while proportion of cycles with total fertilization failure was not statistically different (RR=0.97; 95%CI=0.75-1.25).

Sensitivity analyses, even those after removing studies at high risk of bias or outlier studies, led to similar results and conclusions. Meta-regression showed a significant association between RR of live birth and mean female age, in favour of IVF ($p<0.001$).

Limitations, reasons for caution: The validity of meta-analysis results depends mainly on the quality and the number of published studies available. Indeed, this meta-analysis contains only one randomised controlled trial.

Wider implications of the findings: Conventional IVF should be preferred to ICSI in non-male factor infertility. The use of ICSI should be confined to male factor infertility. This evidence will help improve patient counselling.

Trial registration number: Prospero CRD42019136383

P-116 A prospective randomized sibling-oocyte study of two uninterrupted media systems for culturing blastocysts.

M. Calonge[1], D. Machuca[1], B. Morales[1], C. Troncoso[1], M. Meseguer[2]

[1]IVI Santiago de Chile, IVF Laboratory, Santiago- RM, Chile ;
[2]IVI Valencia, IVF Laboratory, Valencia, Spain

Study question: There is any difference in the probability of getting a live birth when the embryos are cultured in two single step media Vitrolife or Lifeglobal ?

Summary answer: No statistically significant difference in live birth rates between study groups, being uninterrupted embryo culture a feasible strategy with satisfactory outcomes.

What is known already: Currently, commercial media are available in different formulations to support specific stages of embryo development. Culture of embryos is possible under different conditions: whether the media is refreshed, changed or left undisturbed for the 5-6 days of embryo culture. Continuous culture can be interrupted or uninterrupted depending on the renewal of the medium on day 3. Both culture systems have demonstrated excellent clinical outcomes. Very well knows brands like Vitrolife and Lifeglobal have uninterrupted culture media with different specifics formulations but also are different in prices and time of expiration.

Study design, size, duration: We performed a prospective randomized study between November 2017 and December 2018. A total 137 patients from IVI Santiago were included in the study. All mature oocytes were randomized before ICSI to be cultured under either one of the two uninterrupted media formulations tested; Vitrolife or Lifeglobal. Clinical outcomes were compared between study groups.

Participants/materials, setting, methods: Inclusion criteria were patients younger than 37 years and recipient of fresh/vitrified oocytes, with a minimum of 7 MII to ICSI . Exclusion criteria was patients with severe pathologies in sperm sample. All transfer and vitrification of embryos were performed in D5/D6 according to established protocol. Fertilization, blastocyst, pregnancy,

implantation miscarriage and live birth rates were compared between both study groups. Variable significance was analyzed through chi square and logistic regression.

Main results and the role of chance: One hundred and thirty seven study subjects participated in the study, from which 108 underwent an oocyte donation cycle. In total, 1884 MII oocytes were randomized to both media formulations. Nine hundred and thirty four oocytes were microinjected and cultured in Vitrolife medium and nine hundred and fifty in Lifeglobal medium. Fertilization rates were comparable between groups being 74% in Lifeglobal and 78% Vitrolife group. Moreover, no significant differences were obtained in basculation rates (62% Lifeglobal and 54% Vitrolife) , pregnancy rates (67% Lifeglobal and 58% Vitrolife), implantation rates (%62 Lifeglobal and 53%Vitrolife) and miscarriage rates (15% Lifeglobal and 4%Vitrolife). On the other hand, the best 1 or 2 blastocysts based on morphology grading, independent of from which study group coming from were transferred (average 1.16). More blastocysts were selected to transfer in the Lifeglobal group because of their had a better morphology (58% v/s 42%) nevertheless the differences were not statistically significant. Finally in our main objective live birth rate, no statistical differences were found (48% Lifeglobal and 53%Vitrolife)

Limitations, reasons for caution: More studies can be conducted to ingress the number of patients to confirm our findings.

Wider implications of the findings: In agreement with other studies our results confirm that uninterrupted culture media is providing optimal results. As both brands analyzed had comparable results we might suggest each laboratory can select one of those taking into account other important characteristics of management such as prices and durability.

Trial registration number: 1706-SCL-064-MC

P-117 Observations from a multi-centre study using spent culture media for NI-PGT-A

M. Jasper[1], F. Bardan[1], C. Robinson[1], S. Myers[1], M. Fraser[1]

[1]PerkinElmer Health Sciences Australia Pty Ltd, PerkinElmer Health Sciences Australia Pty Ltd, Adelaide, Australia

Study question: Does non-invasive preimplantation genetic testing for aneuploidy (NI-PGT-A) of spent embryo culture media require specific culturing conditions?

Summary answer: Individual laboratories rigorously follow defined embryo culturing protocols to maximise pregnancy outcome and the implementation of non-invasive testing may be possible with minimal protocol changes.

What is known already: Several laboratories are testing the feasibility of using spent embryo culture media for Non-Invasive Pre-implantation Genetic Testing for Aneuploidy (NI-PGT-A). Results published to-date show different rates of concordance with embryo biopsy between laboratories. Variability in the culturing conditions and methodologies being tested may explain the observed difference, as culture conditions are likely to impact the accumulation of both embryonic and contaminating maternal and sperm DNA in spent embryo culture media. As such, optimization of culturing conditions may significantly influence the concordance of results for NI-PGT-A and could assist a controlled evaluation of its use in place of biopsy-based PGT.

Study design, size, duration: NI-PGT using the PG-Seq™ Rapid Non-Invasive PGT kit (PerkinElmer) was performed on spent embryo culture media samples from several laboratories implementing different culturing conditions. The influence of culturing conditions, including culture volume and collection time on concordance between NI-PGT results and embryo biopsy results was analysed and conditions found to have an influence were identified.

Participants/materials, setting, methods: Laboratories provided spent culture media samples for protocol development and/or testing. Once the embryo was removed from the culture droplet, the culture media was collected and stored at -20°C, with ethics approval. Equivalent template volumes of spent embryo culture media were amplified using the PG-Seq™ Rapid Non-Invasive PGT kit protocol, a novel single tube amplification and indexing workflow specifically optimized for NI-PGT-A, and DNA yield was assessed before sequencing.

Main results and the role of chance: Whole genome amplification resulted in the amplification of 77-100% of spent embryo culture media samples provided by the different laboratories (n=9). Ploidy concordance with the embryo biopsy ranged from 23-88% for autosomal chromosomes and 27-63% for sex chromosomes for samples from laboratories using a single-step culturing system (n=2). This was lower than the concordance rates of 45-85% for autosomes and 61-93% for sex chromosomes from laboratories using a two-step culturing system (n=7). Sex chromosome concordance varied between laboratories regardless of the culturing system used, suggesting that embryological processes, such as the technique and accuracy of cumulus cell removal, are important influencers on the accuracy in NI-PGT-A testing. Media droplets ranged in size from 12-70ul, with a mean drop volume of 32ul, and a mean collection time of 60hr (4-144hr). Assisted hatching was perform for some but not all protocols.

Limitations, reasons for caution: Limited numbers of media samples were available for each culturing protocol and researchers performing the data analysis were not blind to the culturing conditions or PGT-A biopsy results. Maternal DNA contamination of media may be underestimated, as it was only possible to detect when the embryo in culture was male.

Wider implications of the findings: Currently there are still many questions on what effect embryology has on the quantity and quality of embryo DNA found in spent culture media. Successful NI-PGT-A may require specific culturing conditions and individual laboratories are best placed to further identify key steps to generate quality concordant results from spent media.

Trial registration number: Not applicable

P-118 Spontaneous blastocyst collapse during pre-vitrification equilibration is related to a lower pregnancy rate: a prospective cohort study

M. Murakami[1], S. Mizumoto[2], K. Tanaka[2], H. Otsubo[2], Y. Nagao[2], T. Kuramoto[3]

[1]Kuramoto Women's Clinic, Research laboratory, Fukuoka, Japan ;
[2]Kuramoto Women's Clinic, IVF laboratory, Fukuoka, Japan ;
[3]Kuramoto Women's Clinic, President, Fukuoka, Japan

Study question: Is there an association between the spontaneous collapse pattern of a blastocyst in an equilibration solution (ES) before vitrification and implantation success?

Summary answer: Ongoing pregnancy rates (PRs) after warmed ET decreased in blastocysts that collapsed completely during equilibration before vitrification.

What is known already: Previous time-lapse studies have found that blastocysts that spontaneously collapse during embryo culture are less likely to hatch in mice and implant in humans, suggesting a negative impact of the collapse on reproductive outcomes. Conversely, it has been shown that artificial shrinkage of human blastocysts before vitrification improves embryo survival and/or pregnancy rates. However, shrinkage is not induced in some vitrification protocols, including ours, as blastocyst viability was already high. To date, no study has been conducted to examine the effect of spontaneous blastocyst collapse during pre-vitrification equilibration on the clinical outcome.

Study design, size, duration: This study included 471 patients undergoing their first autologous IVF/ICSI cycle and freeze-all strategy at the blastocyst stage in our clinic between June 2018 and October 2019. For patients with multiple vitrified blastocysts, embryos for transfer were selected hierarchically based only on morphological scoring (Gardner score) during culture. To minimize bias, only the data from single ETs with Day 5 blastocysts (Score 4, excluding CC) in the first warmed cycle of each patient were analyzed.

Participants/materials, setting, methods: Blastocysts were vitrified-warmed in in-house prepared solutions using Rapid-i carriers. Prior to vitrification, intact blastocysts were equilibrated in 10% ethylene glycol (12 min, 37°C). Their spontaneous collapse patterns were assessed under an inverted microscope before being vitrified in 15% DMSO + 15% ethylene glycol + 0.5 M sucrose. Blastocyst collapse was defined as the separation of the trophectoderm cells from the zona pellucida. Collapsed blastocysts with/without blastocoel cavity were called partially/completely collapsed blastocysts.

Main results and the role of chance: Non-collapsed (NC), partially collapsed (PC), and completely collapsed (CC) blastocyst ETs were 22.9% (108/471), 53.5% (252/471), and 23.6% (111/471) of the ET cycles, respectively. Most embryos (99.6%, 441/443) survived warming. PRs per ET tended to decrease as blastocysts progress from one collapse pattern to another. In this study, the ET cycles were divided into NC/PC and CC groups to facilitate data

analysis. Maternal age was similar between the groups. Clinical PRs per ET were 61.7% (NC/PC) versus 51.4% (CC) (odds ratio (OR): 1.52, 95% confidence interval (CI): 0.97-2.39, *P* = 0.061). Ongoing PRs per ET were 55.8% (NC/PC) versus 37.8% (CC) (OR: 2.07, 95% CI: 1.31-3.30, *P* = 0.001). A logistic regression analysis was performed on the ongoing PRs to account for the effects of some possible confounding factors, including the complete blastocyst collapse, blastocyst morphology, MII oocyte number, and maternal characteristics (age, parity (yes/no), smoking (yes/no), and BMI). In addition to the maternal age (years, OR: 0.89, 95% CI: 0.84-0.94, *P* < 0.001) and blastocyst morphology (good (4AA/4AB/4BA) versus not, OR: 1.60, 95% CI: 1.09-2.36, *P* = 0.017), the blastocyst collapse (yes versus no) had a significant effect on the results (OR: 0.48, 95% CI: 0.31-0.77, *P* = 0.002).

Limitations, reasons for caution: Cryoprotectant concentration of our ES is relatively low. Thus, when considering the collapse pattern as a criterion for selecting embryos for transfer, caution is needed as ES osmolarity can influence blastocyst shrinkage. Further studies with more participants are required to confirm the results.

Wider implications of the findings: This is the first study to demonstrate a relationship between spontaneous blastocyst collapse during pre-vitrification equilibration and a decreased pregnancy rate. Studying the collapse pattern may assist in selecting the most viable blastocysts after warming and increase IVF success rates.

Trial registration number: UMIN000039264

P-119 Influence of culture media on embryo developmental pattern and ploidy status: a sibling oocyte study.

A. Abdala[1], I. Elkhatib[1], A. Bayram[1], A. Arnanz[1], A. El-Damen[1], B. Lawrenz[1,2], H.M. Fatemi[1], N. De Munck[1]

[1]IVIRMA Middle East, Fertility Clinic, Abu Dhabi, United Arab Emirates ; [2]Women´s University Hospital Tuebingen, Obstetrical Department, Tuebingen, Germany

Study question: Is there a difference in embryo development and euploidy rate between blastocysts cultured in continuous culture media (CCM) or sequential culture media (SCM)?

Summary answer: While the blastulation on day 5 is increased in CCM, the usable blastocyst and euploidy rates are not significantly different between CCM and SCM.

What is known already: Culture medium is fundamental for *in vitro* blastocyst development. While CCM supplies all nutrients that embryos need to grow ("let the embryo choose"), SCM mimics physiological conditions ("back to nature"). Many studies evaluated whether CCM improves clinical outcomes as compared to SCM, hence, comparisons failed to identify a superiority. Recent data suggest that euploidy rates may be affected by culture media, however, non-consensus has been reached due to the variability of experimental designs, underpowered studies and heterogenicity that might affect outcomes. The lack of standardization of the comparative studies drives to controversial conclusions of which culture media improve euploid outcomes.

Study design, size, duration: A single center observational cohort study was performed between September 2018 and March 2019, including 1452 mature oocytes (MII). Patients who underwent preimplantation genetic testing for aneuploidies (PGT-A) with at least four fresh autologous MII inseminated by ICSI were included. Severe male factor was excluded. According to clinical practice, sibling MII oocytes were randomly split between two media: 751 in Global Total LP (CCM) and 701 in Sage cleavage and blastocyst media (SCM).

Participants/materials, setting, methods: Zygotes were cultured up to day 7 with both media refreshed on day 3 under 6% CO_2, 5% O_2 and 89% N_2, pH=7.2-7.4. Fertilization, cleavage, D5 blastulation, usable blastocyst (total blastocysts biopsied/total blastocysts formed) and euploidy rates were recorded. Blastocyst expansion, inner cell mass (ICM) and trophectoderm (TE) quality were graded according to Gardner´s scoring system, prior TE biopsy on day 5, 6 or 7. TE samples were analyzed for PGT-A by next generation sequencing.

Main results and the role of chance: Patient mean age was 34.3±6.0 years old. No differences were observed in fertilization and cleavages rates (77.4% vs 75.5%, p=0.468, and 97.6% vs 99.1%, p=0.089 for CCM and SCM, respectively). Total number of blastocysts formed was similar between CCM and SCM (3.4±5.5 vs 2.9±2.3, p=0.075). However, blastulation rate on day 5 was significantly higher for embryos cultured in CCM (72.2% vs 63.1, p=0.017). The usable blastocyst rate was not significantly different between the two groups compared (76.5% vs 79.3%, p=0.448 for CCM and SCM). From a Poisson regression model adjusted for confounding factors, no differences were observed in euploidy rate between CCM and SCM (OR=2.4387, CI 95%: 0.93-6.38, p=0.0699 for CCM and OR=1.1791, CI 95%: 0.94-1.48, p=0.1572, for SCM). Only patients age was associated with the euploidy rates, regardless the type of culture medium (OR=0.9494, CI 95%: 0.93-0.97, p<0.001). A linear mixed model was performed controlling for confounding factors and a significant increase in toP-quality blastocysts (ICM= A or B and TE=A) was found when embryos were cultured in SCM (OR=-0.2545, p=0.814 for CCM and OR=0.5070, p=0.036 for SCM).

Limitations, reasons for caution: The current results are based on a retrospective observational study.

Wider implications of the findings: Under controlled conditions, *in vitro* outcomes are comparable when using a CCM or SCM, although quality of blastocysts can be improved when embryos are cultured in SCM. As expected, patient's age has the most important impact on euploidy rate.

Trial registration number: None

P-120 Embryos that divided chromosome correctly develope to the morphologically-good blastocyst in spite of abnormal cytokinesis at 1st mitosis

S. Hashimoto[1], Y. Nakaoka[2], Y. Morimoto[3]

[1]Osaka City University Graduate School of Medicine, Reproductive Science Institute, Osaka, Japan ; [2]IVF Namba Clinic, IVF Namba Clinic, Osaka, Japan ; [3]HORAC GrandFront Osaka Clinic, HORAC GrandFront Osaka Clinic, Osaka, Japan

Study question: Why do human embryos normally develop after abnormal cytokinesis at 1st mitosis?

Summary answer: Only embryos that underwent correctly chromosomal division developed to the morphologically-good blastocyst in spite of abnormal cytokinesis at 1st mitosis.

What is known already: It has been shown that a live birth from embryos which underwent abnormal cytokinesis at 1st mitosis. During abnormal cytokinesis, there are two main patterns in chromosomal division, such as dual spindle formation without syngamy and multipolar spindle formation with syngamy. It's unlikely to believe these abnormally chromosome-separated embryos could develop to babies. There is the potential to include morphologically-abnormal cytokinesis without abnormal chromosome partitioning, such as fragmentation.

Study design, size, duration: This study was approved by the ethical committee of the Japan Society of Ob/Gy. We assessed chromosomal behavior during 1st mitosis in human zygotes and their development in vitro using 140 donated zygotes. Pronuclear ova were donated from infertility couples after obtaining their informed consent.

Participants/materials, setting, methods: Donated pronuclear ova were injected with a mixture of mRNAs encoding EGFP-EB1 and mCherry-histone-H2B. Dynamic changes of their chromosomes were monitored continuously using a confocal microscope inside an incubator every 15 min for 24 h. After live imaging, morphological changes of embryos were recorded by a conventional time-lapse system using a light microscope.

Main results and the role of chance: To directly examine possible idea for inclusion of embryos without abnormal chromosome partitioning into abnormal cleavage, we observed chromosomal behavior during 1st mitosis using live cell imaging system. One hundred-forty zygotes underwent 1st mitosis after RNA injection. Abnormal cytokinesis at 1st mitosis was observed in 45 zygotes (32%). In 12 zygotes, normal chromosome division was observed under a confocal microscope (27%) despite abnormal cytokinesis. Three zygotes developed to the blastocyst stage from normally chromosome-divided embryos (25%). One of these blastocysts were categorized as morphologically-good (4BB) by Gardner's strict criteria. Monitoring of chromosomal behavior at 1st mitosis using a confocal microscope enables us to distinguish embryos which underwent correct chromosomal division in spite of abnormal cytokinesis. The data of the present study suggest that embryos which underwent chromosomal division correctly could develop to live babies in spite of abnormal cytokinesis at 1st mitosis.

Limitations, reasons for caution: It is not ethically permitted in clinical fields to monitor the chromosomal behavior at 1st mitosis of human embryos under a confocal microscope. Further studies are required to detect the difference between correct and incorrect chromosomal division using a light microscope.
Wider implications of the findings: Live imaging using a confocal microscope inside an incubator yielded information about chromosome behavior during preimplantation embryo development that were unobservable by a time-lapse system using a light microscope. Live imaging is expected to play an important role in understanding chromosomal aberrations during pre-implantation embryo development.
Trial registration number: Japan Society of Obstetrics and Gynecology 112

P-121 Prevalence and predictive factors for complete fertilization failure in in vitro fertilization treatment cycles: a retrospective analysis of a large-scale nationwide database study

T. Takahashi[1], K. Ota[1], H. Mizunuma[1]

[1]Fukushima Medical University, Fukushima Medical Center for Children and Women, Fukushima, Japan

Study question: What is the prevalence of and what are the risk factors for complete fertilization failure (CFF) in patients undergoing *in vitro* fertilization (IVF) treatment cycles.
Summary answer: The prevalence of CFF was 13.2% and the major predictive factor for IVF cycles with CFF was a retrieved oocyte number.
What is known already: It is reported that the rate of CFF in IVF treatment cycles ranges from 5 to 15%. A more accurate indicator of prevalence is unknown, and the predictive factors for CFF in IVF cycles based on real-world data are unclear.
Study design, size, duration: A retrospective cohort study was performed, based on 1404648 treatment cycles in registered assisted reproductive technology (ART) data from the Japan Society of Obstetrics and Gynecology between 2007 and 2012.
Participants/materials, setting, methods: We analyzed data from 220,065 fresh cycles of IVF when one or more oocytes were retrieved. An instance of CFF was defined as zero fertilized oocytes obtained. The IVF cycles with CFF or with one or more oocytes fertilized were compared to determine predictive factors for CFF using multivariate logistic regression analyses. The area under the receiver operating characteristic (ROC) curve was used to assess the discriminative ability of the logistic models.
Main results and the role of chance: The total percentage of CFF in IVF cycles was 13.2% (29076/220065). On multivariate analysis, patient age (adjusted odds ratio [aOR]: 1.00, 95% confidence interval [CI]: 1.00-1.01, p<0.01), oocyte number (aOR: 0.78, 95% CI: 0.77-0.79, p<0.01), sperm motility (aOR: 0.99, 95% CI: 0.98-0.99, p<0.01), and male factor infertility (aOR: 1.43, 95% CI: 1.38-1.49, p<0.001) were independent predictive factors of CFF. The percentages of CFF cycles with one, two, three, four, and five oocytes retrieved were 34.2%, 15.3%, 8.6%, 6.0%, and 4.6%, respectively. The efficacy of oocyte number and sperm motility for predicting CFF were evaluated using ROC curves. The areas under the curves for oocyte number and sperm motility were 0.79 and 0.59, respectively. Setting the threshold at 2 for oocyte number offered the best compromise between sensitivity (0.66) and specificity (0.80).
Limitations, reasons for caution: As cases with serious semen abnormalities may have undergone intracytoplasmic injection, a selection bias cannot be ruled out in the IVF treatment cycles analyzed.
Wider implications of the findings: This study showed that collecting a large number of retrieved oocytes may prevent CFF. These results provide information that may be useful for counseling patients before their undergoing ART treatments.
Trial registration number: not applicable

P-122 Investigation of transfer results of human embryos which are vitrified and thawed in cleavage, morula and blastocyst stages

C. Korkmaz[1], U.G. Yıldız[2], U. Fidan[2], B. Baykal[2], S.T. Ceyhan[2], E. Agacayak[2]

[1]University of Health Sciences- Gülhane Training and Research Hospital Ankara-Turkey, IVF Center, Ankara, Turkey ;
[2]University of Health Sciences- Gülhane Training and Research Hospital, IVF Center, Keçiören, Turkey

Study question: What is the rates of clinical pregnancy after the transfer of vitrified and thawed human embryos on Day 3, Day 4, and Day 5 of embryonic development.
Summary answer: Higher rates of pregnancy were determined with the freezing of human embryos on Day 4 and the transfer of those embryos on Day 5.
What is known already: One of the most important stages in clinical infertility treatment today is frozen embryo transfer. The cryopreservation of human embryos allows us to increase the potential of conception for any in vitro fertilization (IVF) cycle to its highest level and to prevent the waste of embryos. Vitrification, which is currently used to freeze human oocytes and embryos at the pronucleus, cleavage, morula, and blastocyst stages, is a simple, low-cost, and effective method.However, there is no consensus regarding which stage of embryonic development is most suitable for vitrification with maximum development after the embryo is thawed.
Study design, size, duration: 148 embryo transfer cycles, using embryos frozen with the closed vitrification method, thawed during the three-year period between January 2016 and December 2018 were investigated. Results of the embryos frozen were compared in three groups based on the day they were frozen Day 3, Day 4, or Day 5. This study is the first one in which pregnancy results are compared following freezing and thawing of human embryos in the cleavage, morula, and blastocyte stages.
Participants/materials, setting, methods: 128 patients, and 148 cycles belonging to this infertile patients, were included in our study. Male majority factor cases were excluded from the study. Closed vitrification method were used during the study. 17.4% of patients' embryos were frozen on Day 3, 55.6% on Day 4, and 27% on Day 5.The clinical pregnancy and ongoing pregnancy results were examined, after thawing.
Main results and the role of chance: The clinical pregnancy and ongoing pregnancy results were examined, after thawing, in all groups according to their infertility reasons, with no significant difference observed in terms of clinical pregnancy and ongoing pregnancy rates (p:0.061, p:0.493). 20% of the embryos frozen on Day 3 were also transferred on Day 3; 52% were transferred on Day 4; and 28% were transferred on Day 5. 26.2% of the embryos frozen on Day 4 were transferred on Day 4, and 73.8% were transferred on Day 5. All embryos frozen on Day 5 were transferred the same day. Clinical pregnancy and ongoing pregnancy rates were observed to be significantly higher in the results of embryos frozen on Day 4 and transferred on Day 5, according to the transfer day (clinical pregnancy rates were 28.5% on day four and 49.1% on day five)
Limitations, reasons for caution: There is a need for comparative studies on vitrification of human embryos at different stages with a larger patient population.
Wider implications of the findings: This study is the first one in which pregnancy results are compared following freezing and thawing of human embryos in the cleavage, morula, and blastocyte stages. Higher pregnancy rates can be achieved by transferring, in the blastocyst stage, embryos that were frozen and thawed in the morula stage.
Trial registration number: 19/48

P-123 The expression of Anti-Müllerian hormone receptor type II (AMHRII) in human oocytes from the in vitro fertilization programme and their maturation with recombinant AMH

J. Bedenk[1], E. Vrtačnik-Bokal[1], N. Jančar[1], K. Geršak[1], I. Virant-Klun[1,2]

[1]University Medical Centre Ljubljana, Department of Obstetrics and Gynaecology, Ljubljana, Slovenia ;
[2]University Medical Centre Ljubljana, Centre for Clinical Research, Ljubljana, Slovenia

Study question: Is AMHRII receptor present in the immature and mature non-fertilized oocytes from the IVF programme; can recombinant AMH affect oocyte maturation in vitro?
Summary answer: AMHRII receptor is more expressed in immature than mature oocytes and immature oocytes can be matured in vitro in the presence of recombinant AMH only.

What is known already: AMHRII, a key receptor for AMH, is expressed in Sertoli and Leydig cells in testes and in granulosa and theca cells in the ovaries. This receptor is crucial for the function of Anti-Müllerian hormone (AMH) in follicular cells, especially as a regulator of growing ovarian follicles. Despite this, we could not find any studies on the expression of AMHRII receptor in human oocytes. Therefore, we decided to analyze the oocytes for AMHRII receptor expression and mature the oocytes in vitro with recombinant AMH in the maturation medium to perhaps demonstrate the direct action of this hormone on oocytes.
Study design, size, duration: During two years, we included two groups of oocytes – 24 immature (GV) and 24 mature (MII) oocytes to perform immunocytochemistry for expression of AMHRII and elucidate whether the maturity stage of oocytes has an effect on expression of AMHRII receptor. In the next step, 15 immature and 15 mature oocytes were analysed on expression of *AMHRII* gene. Immature oocytes (n=15) were matured in vitro in the presence of recombinant AMH to test its activity.
Participants/materials, setting, methods: We used the oocytes from patients included in the in vitro fertilization programme (ICSI) after their written informed consent. Immunocytochemistry was performed using the anti-AMHRII antibody; fluorescence was quantified with Fiji software. Gene expression analysis for *AMHRII*, *ZP1*, *ZP2*, *ZP3*, *ZP4*, *BMP4* and *GDF9* was performed using qPCR after superamplification, as well as in vitro maturation of oocytes with recombinant AMH (R& DSystems 1737-MS, 100 ng/mL) alone in the maturation medium of MediCult IVM System.
Main results and the role of chance: The AMHRII receptor was expressed in both immature and mature ocytes, mostly in a spotted pattern through the whole cell membrane, as well as in the zona pellucida, but in a much lower quantity. There was also accumulation of AMHRII protein around the genetic material (germinal vesicle) in the immature oocytes, as revealed by immunocytochemistry. The qPCR analysis confirmed the expression of gene *AMHRII* as well as the co-acting genes related to zona pellucida (*ZP1*, *ZP2*, *ZP3*, *ZP4*) and development (*BMP4* and *GDF9*) in immature and mature oocytes, which may reflect the potential function of AMHRII in the oocytes. We also proved that *AMHRII* gene was expressed 10x more in immature oocytes than in mature oocytes (P=0,006; Student T-test). All immature oocytes matured in vitro in the presence of recombinant AMH alone (without FSH and HCG) in the maturation medium. Our preliminary data show that AMH does not act only on the follicular cells but may also act on the oocyte directly.
Limitations, reasons for caution: The limitations of this research may be: impaired quality of mature oocytes which did not fertilize after ICSI and a relatively low number of oocytes. A proportion of them degenerated during the immunostaining procedure. This research is still ongoing to increase the number of oocytes.
Wider implications of the findings: Based on our data, we assumed that AMH, in addition to follicular cells, may directly act on oocytes in vitro. This implies a new understanding of the human oocyte maturation process. Further research will show whether recombinant AMH can improve the process of human oocyte maturation in vitro.
Trial registration number: Republic of Slovenia National medical ethics committee, trial number 0120-546/2018/6

P-124 ICSI versus IVF on sibling oocytes: The real efficacy comparing laboratory results and clinical outcomes

S. Chamayou[1], R. Lombardi[1], C. Ragolia[1], C. Alecci[1], G. Storaci[1], R. Sapienza[1], S. Romano[1], C. Cardea[1], A. Liprino[1], E. Maglia[1], A. Guglielmino[1]

[1]U.M.R. Soc. Coop. a.r.l., Unità di Medicina della Riproduzione, Sant'Agata li Battiati-Catania, Italy

Study question: ICSI versus IVF : which technique is more efficient based on *in vitro* results and clinical outcomes in absence of severe male factor? Conclusions from sibling oocytes study.
Summary answer: Despite of the highest fertilization rate in ICSI, IVF-derived embryos have a higher implantation rate compared to ICSI-derived embryos.
What is known already: The ICSI procedure has been invented to treat severe male infertility and full fertilization failure after conventional IVF. Nowadays ICSI tends to be applied in every case to ensure fertilization as reported in the latest report from the European IVF-monitoring Consortium (2014) where ICSI resulted applied in 71.3% of the fresh treatments.
Study design, size, duration: The present study includes 308 couples who underwent assisted reproduction treatment at UMR-centre HERA between October 2009 to April 2019. The couples were counseled for *in vitro* treatment and were proposed to perform ICSI and IVF on sibling oocytes. In none of the cases infertility was due to severe male factor or previous fertilization failure after *in vitro* technique. The average age for female patients was 33.4.
Participants/materials, setting, methods: The patients underwent ovarian stimulation using GnRH agonist or antagonist protocol. After oocytes retrieval, oocytes-cumulus complex were randomly divided into two groups. Oocytes were simultaneously fertilized by ICSI and IVF with the same semen sample. Fertilization and embryo development were assessed by time-lapse monitoring. Embryos were transferred on the same cycle and surplus blastocysts vitrified for further transfer. Fertilization rate, embryo kinetic and embryo implantation rates were compared between ICSI and IVF.
Main results and the role of chance: On 3,400 oocytes collected, 1,270 were fertilized by ICSI and 1,035 by IVF. Fertilization rate was statistically higher in ICSI group compared to IVF group (76.3% in ICSI versus 58.6% in IVF, $p < 0.0001$). There were no statistically significant differences in cleavage rate (98.7% in ICSI versus 98.7% IVF; $p > 0.05$), embryonic quality in GII (39.7% versus 41.8%) and the rate of usable embryo on total produced embryos per technique (49.4% versus 46.2%) between ICSI and IVF ($p>0.05$). The rates of clinical pregnancy (46.6% vs. 46.8%), of abortion (12.7% vs. 13.3%) were also comparable ($p> 0.05$). Implantation rate was statistically higher for embryos from IVF compared to the sibling embryos produced in ICSI (42.6% versus 55.2%, $p <0.05$). Embryo kinetic was faster in ICSI compared to IVF.
Limitations, reasons for caution: Not all vitrified surplus blastocysts have been thawed and transferred. Consequently cumulative pregnancy rate is incomplete.
Wider implications of the findings: ICSI may induce adverse event on embryo competence. In case of absence of indication for ICSI, IVF should be preferred as fertilization method.
Trial registration number: xx

P-125 Identification of the optimal puncture position on oolemma without degeneration in Piezo-ICSI using image analysis: a pilot study

S. Mizuta[1], T. Maekawa[2], Y. Kinishi[3], H. Matsubayashi[2], K. Kitaya[1], T. Takeuchi[2], Y. Hata[3], T. Ishikawa[1]

[1]Reproduction Clinic Osaka, Reproductive Medicine, Osaka, Japan ; [2]Reproduction Clinic Tokyo, Reproductive Medicine, Tokyo, Japan ; [3]University of Hyogo, Graduate School of Simulation Studies, Kobe, Japan

Study question: Is it possible to identify the optimal puncture position on oolemma which may not cause degeneration in Piezo-ICSI using image analysis?
Summary answer: Visualizing the likelihood of unintentional membrane rupture using image analysis can identify positions that result in lower risk of oocyte degeneration following Piezo-ICSI.
What is known already: Oocyte degeneration may take place in Piezo-ICSI as a result of unintentional membrane rupture in the puncturing process. Identifying the appropriate puncturing position may decrease the likelihood of membrane rupture and thus degeneration, but this cannot be done visually. Image feature analysis is generally used to represent the useful features such as color, brightness, and contour. Among capturing image features, Local Binary Patterns (LBP) can efficiently summarize the local structures of images, and it has been applied in texture analysis in various fields including face recognition. There were no studies identifying the optimal puncture position using image analysis.
Study design, size, duration: We retrospectively performed the LBP methodology to analyze the moving images of 131 oocytes following Piezo-ICSI between August 2018 and January 2019. These oocytes were categorized as either having undergone unintentional rupture (UR: n = 30) or no rupture (NR: n = 101).
Participants/materials, setting, methods: LBP values were calculated in the analysis region centered around the puncture position. Median values for UR and NR were calculated to select an effective pattern for rupture evaluation from the 256 shape patterns acquired by LBP. After choosing the three effective patterns with hierarchical clustering, we employed Ward's hierarchical cluster analysis method and calculated the Euclidean distance between the cluster barycenter and each data point to define an index indicating membrane rupture implausibility.

Main results and the role of chance: Two clusters, the cluster A and B, were classified from hierarchical clustering. Following ICSI, 2 out of 27 oocytes from the cluster A and 28 out of 104 from the cluster B resulted in UR, indicating that the cluster A strongly represented NR group and the cluster B weakly represented UR group, with the sensitivity as 0.93. A significant difference between the UR and the NR group was reported from the Euclidean distance calculations between the barycenter of Cluster A and each data point (P = 0.001), where data showed a longer distance from the barycenter amongst the UR group and a shorter distance in the NR group. All degenerated oocytes after ICSI procedure were from UR (27.7%, 8/30). Of these, two were from the cluster A (100%, 2/2) and six were from the cluster B (21.4%, 6/28), while the 2PN rate was 0% and 67.9% in the clusters A and B, respectively. On the other hand, no degeneration was observed from the NR group, and the 2PN rate was 84.0% and 81.6% in the clusters A and B, respectively. Fertilization and degeneration results in NR were significantly better than UR groups.
Limitations, reasons for caution: This was a retrospective pilot study with a small sample size, and this was a single-center study. Moreover, this study did not include embryonic development and clinical outcomes. Further prospective studies in large samples size are needed.
Wider implications of the findings: We can retrospectively classify UR or NR from the shape feature of the oolemma by the image analysis with AI. If we could recognize optimal puncture position by visualizing shape features of the oolemma in real-time, we could reduce degeneration of the oocytes after Piezo-ICSI.
Trial registration number: not applicable

P-126 Day of blastocyst freezing is a better prognostic factor than morphology for livebirth in single frozen embryo blastocyst transfer

M.W. Lui[1], C. Lamb[2], D. Thong[3], S. Pickering[2], K.J. Thong[2]

[1]Queen Mary Hospital- Hong Kong, Obstetrics and Gynaegolocy, Hong Kong, Hong Kong ;
[2]Royal Infirmary of Edinburgh, Edinburgh Fertility and Reproductive Endocrine Centre, Edinburgh, United Kingdom ;
[3]Statistician, independent researcher, Edinburgh, United Kingdom

Study question: Is the day of blastocyst freezing or blastocyst morphology a better prognostic factor for livebirth in single frozen embryo transfer (FET)?
Summary answer: Day-5 blastocyst is associated with higher livebirth rate than day-6 blastocyst, even with a better morphological scoring at freeze.
What is known already: Assessment of development potential is essential in reducing the pregnancy interval in the era of single embryo transfer and optimizing the livebirth rate from treatment. The rate of blastocysts development (day-5 or day-6 blastocysts) (1) and morphological assessment (2, 3) have both been identified as significant prognostic factors for livebirth in blastocyst(s) transfer. However, we are uncertain which of the parameters is a better prognostic factor for livebirth rate for women undergoing FET.
Study design, size, duration: A retrospective study was carried out for all single blastocyst frozen transfer from January 2015 to December 2018 in a single fertility centre. Cases requiring extended culture after thawing and incomplete data were excluded. The cases were identified from the centre's computerized data. Morphological assessment was carried out using the Gardner and Schoolcraft Grading System (4). Good quality embryos were defined as 1-6AA, 3-6AB or 3-6BA.
Participants/materials, setting, methods: A total of 1541 single blastocyst transfers were carried out during the study period and 1162 transfers were included in the analysis. Chi-square testing and binary logistic regression of live birth rate against: age of women at freezing, interval of freezing, embryo quality after thaw and change in quality from freezing to thawing were performed. Women with morulas after thaw were excluded in the analysis using logistic regression.
Main results and the role of chance: The mean age of women at freezing and duration of freezing were 34.0 +/- 3.9 years and 0.9 +/- 1.3 years respectively. The overall livebirth rate was 31.4%. The livebirth rate was the highest for good quality day-5 blastocysts of 39.4%, followed by 25.2% in poor quality day-5 blastocysts. It was lower for good quality day-6 blastocysts of 22.5% and 15.9% in poor quality day-6 blastocysts (p <0.001 for differences and trend).

Logistic regression found the followings to be significant: Age at freezing ($p < 0.001$, OR 0.939, 95% CI 0.908 - 0.971), Day 5 vs Day 6 transfer ($p < 0.001$, OR 2.060, 95% CI 1.443 - 2.978), trophectoderm score of A upon thawing ($p = 0.01$, OR 1.482, 95% CI 1.100 – 1.997), and the expansion score upon thawing ($p < 0.001$ overall via likelihood ratio Chi-square test). Embryos with an expansion score of 1 have 75.7% lower odds of live birth than embryos with grade 6 expansion score (OR 0.243, 95% CI 0.085 – 0.722). The score for inner cell mass was not found to be significant.
Limitations, reasons for caution: Other potential covariates including body mass index, parity and aetiology of infertility were not included in the analysis. Other morphokinetic factors such as abnormal cleavage may have been included into selection process for freezing and thawing, but were not included in the analysis.
Wider implications of the findings: The study provides a simple decision support tool for embryologists to decide on which frozen blastocysts to transfer. Morphological features such as trophectoderm grade and expansion score are predictors of livebirth. Wherever possible, a day 5 blastocyst should be transferred in preference to a day 6 blastocyst.
Trial registration number: not applicable

P-127 Understand chromosomal self-correction of human embryo with non-invasive genomic test

J. Yan[1], M. Li[1], L. Xiang[1], X. Wu[1], Y. Li[1]

[1]The First People's Hospital of Yunnan Province-, Reproductive Medical Center-, Kunming, China

Study question: Can mosaic embryo commit self-correction or elimination process during development? Are there differences between pre-implantation (D1~D5) and post-implantation stage (D5~D14)?
Summary answer: Chromosomal self-correction could be observed in both pre- and post-implantation stage. In this research, most of the aneuploidy cells decreased before Day 5 post fertilization.
What is known already: Chromosomal mosaicism in human pre-implantation embryos has been described, and is regarded as a significant factor that contributes to unsuccessful embryo implantation and spontaneous abortion. It is reported in cleavage stage, the mosaic embryo is up to 70%. and in blastocyst the ratio reduces to about 30%. Current hypothesis is during the development embryo excluded the aneuploidy cells for self-correction. However, lack of proper approaches, the quantitatively reports are still rare. The post-implantation stage situation is also elusive.
Study design, size, duration: From 2019~2020, 36 frozen embryos from 23 patients (age 22~34 years), who determine to donate their embryos after successful pregnancy with informed consent in Reproductive medical center, the first people's hospital of Yunnan province,China.
Participants/materials, setting, methods: For each cleavage embryo, 2~3 blastomeres are tested and the embryo was continually cultured to blastocyst. and collected the medium In Day5, 1~2 TE and 1 ICM are biopsied. For mimicing post-implantation embryo in vitro, three-dimensional blastocyst-culture system(Nature, 2010) is adopted. This method allowed the development of 3D structure of embryo and mimic the in vivo situation better. The culture medium are collected in Day8/10/2. Medium and embryo are sequenced with NGS (Yikon).
Main results and the role of chance: In our study, 92% (297/323) samples are successfully amplified. Data from ICM are considered as represented result for the embryo. In pre-implantation stage, mosaicism portion over 20% is included, and 24% (6/25) of the embryos carrying at least one mosaic chromosome. 72% (17/25) results from mediums are at least consistent with TE or ICM. In pre-implantation stage, the consistent of each blastomeres are 83% (55/66) TE and ICM are 86.3%(19/22). 16%(4/25) self-correction (CNV in blastomeres but not in TE/ICM or the mosaic ratio decreased) and 4%(1/25) novel chromosomal aneuploidy (CNV in TE but not in blastomeres) are observed. 8%(2/25)aneuploidy is detected in medium but not in either blastomere or TE/ICM.

In post-implantation stage, 81.8%(9/11) are developed embryos carrying consistent chromosomal situation. One duplication of chromosome 14 is observed in Day8 but not in Day10 and Day12(1/11) .
Limitations, reasons for caution: The size of the research is still small; the post-implantation and pre-implantation embryos are not the same batch; some

of the donated embryos are not from the ICSI cycle, potentially maternal contamination could not be excluded.
Wider implications of the findings: Non-invasive chromosomal test provides us powerful tool to study the genomic change. We find more mosaicism in medium but not in biopsy, and bsed on current study size, most self-correction could be observed before Day5, for post-implantation stage, more evidence are required.
Trial registration number: NSFC 31700798

P-128 Prospective comparative study between two commercial single culture media using sibling donor oocytes collected from randomized ovaries

C. MIRET LUCIO[1], M. Benavent[1], M. Escriba[1], A. Garcia[1], D. González[1], N. Borges[2], G. Calderon[2], J. Crespo[3], J. Teruel[1]

[1]Equipo Médico Crespo, IVF Lab, Valencia, Spain ;
[2]Embryotools, IVF Lab, Barcelona, Spain ;
[3]Equipo Médico Crespo, Reproductive Medicine, Valencia, Spain

Study question: To compare embryo development and clinical outcomes between two commercial single culture media using sibling donor oocytes collected from randomized ovaries
Summary answer: Our study suggests that different commercial single media used uninterruptedly can yield different blastocyst developmental rates, depending on their formulation and/or environmental culture conditions
What is known already: In recent years, there has been a renewed interest in the use of single media to support human embryo culture *in vitro*. These media are based on a single formulation designed following the principle of allowing developing embryos to choose the nutrients according to their needs. An increasing number of single media have now become commercially available and have been swiftly introduced in IVF laboratories worldwide. Whether all single media are equally suitable for the continuous (one-step) culture of human embryos remains unclear, as recent studies suggest that the formulation, laboratory environment and culture conditions may result in different outcomes
Study design, size, duration: This is a single-center prospective study performed between February and December 2019 that included 84 donors and 94 recipients. Ovaries were randomized using a computer-generated randomization list. Sibling oocytes collected from each randomized ovary were processed and cultured in two commercial single media (A and B). The corresponding series media (fertilization, handling or culture) offered by each brand was used to perform all procedures involving oocytes and sperm samples of the two experimental arms
Participants/materials, setting, methods: Oocytes were injected by ICSI and then cultured individually in miniGPS dishes (LifeGlobal) prepared with either single medium A or B (25ul medium/well) at 37°C, in an atmosphere of 6-7% CO2/5% O2/88-89% N2 of a dry atmosphere incubator (IVFcube, Astec). Laboratory conditions, such as temperature, humidity and volatile organic compounds (VOC) levels were monitored continuously (Octax Log&Guard, Vitrolife) during the study period and pH measured in a weekly basis in the two single media used
Main results and the role of chance: The pH average values were similar in both media (medium A 7.28±0.06 vs medium B-7.27±0.05), and the mean value of VOCs 0.032 ppm. A total of 1103 MII oocytes were injected by ICSI (A, n=558 and B, n=545). The proportion of fertilized oocytes was identical between the two media (A:83.9% and B:79.4%), but oocyte degeneration rate post-ICSI was significantly lower in group A (3.6% vs 7.3%; p=0.0059). The mean number of embryos that reached the blastocyst stage cultured in the single medium A (64.3%) was significantly lower (p=0.004) than that obtained in medium B (73,2%). Similarly, a higher proportion (p=0.031) of blastocysts suitable for clinical use (transferred or cryopreserved) was obtained in medium B (61%) compared to medium A (53.8%).

Ninety-four patients had an embryo transfer on day 5/6 with either fresh or cryopreserved blastocysts cultured in single medium A (n=39) or B (n=56), with a mean number of 1.2 blastocysts transferred/patient in the two groups. No differences were found in terms of clinical pregnancy (69.2% and 64.3%, respectively) or implantation rates (67.4% and 56.1%, respectively) between both groups. Miscarriage rates were similar between group A (18.5%) and group B (12.5%). Three mixed transfers were excluded from the interpretation of results
Limitations, reasons for caution: Continuous embryo culture in single media largely relies on optimal laboratory conditions and a series of osmolality or atmospheric fluctuations, accumulation of volatile organic compounds or ammonium built-up may negatively affect the culture environment. Future research is needed to compare reproductive outcomes in RCT performed with patient's own oocytes
Wider implications of the findings: The present study suggests that commercial single culture media when used in a continuous (one-step) approach may result in different outcomes in terms of proportion of suitable blastocysts for clinical use. Single media formulation, laboratory conditions and the culture environment should be properly validated independently on each laboratory conditions
Trial registration number: Not applicable

P-129 pregnancy is significantly correlated with the blastocyst width and area: a time-lapse study.

R. Sciorio[1], D. Thong[2], K.J. Thong[3], S. Pickering[3]

[1]Royal Infirmary of Edinburgh, Edinburgh Assisted Conception Programme- EFREC, Edinburgh- Scotland, United Kingdom ;
[2]RIE, Efrec, Einburgh, United Kingdom ;
[3]RIE, Efrec, Edinburgh, United Kingdom

Study question: Does blastocyst width and area affect pregnancy outcome in IVF/ICSI cycles?
Summary answer: Clinical pregnancy rate (CPR) is significantly higher for blastocysts with a larger width and area, which was confirmed by logistic regression.
What is known already: In order to maintain pregnancy rates following single embryo transfer, optimisation of embryo culture and selection is vital. Time-lapse monitoring (TLM) has the potential to play a crucial role by providing sequential images of embryo development and minimal disturbance, thereby increasing the probability of selecting embryos with high implantation potential. However, transfer of good quality embryos does not always lead to successful pregnancy. Therefore, in this study *morphometric* evaluation was performed. We used a single assessment of blastocyst area and maximum width to evaluate if these parameters correlated with pregnancy outcomes.
Study design, size, duration: A retrospective analysis of 664 patients who had eSET was carried out between April 2014 and August 2019. Embryos were cultured individually in 6.0% CO_2, 5.0% O_2, 89.0% N_2, using single step medium (G-TL™ Vitrolife, Göteborg, Sweden) in the time-lapse incubator (Embryoscope, ES-D, Vitrolife).
Participants/materials, setting, methods: All embryos were evaluated using calibrated annotation tools of the EmbryoViewer. Drawing tools were used to measure specific variables such as the maximum blastocyst width and blastocyst area. Data obtained was assessed in terms of CPR at 7 weeks gestation. Statistical analysis was performed with Mann-Whitney *U*-test and Logistic Regression.
Main results and the role of chance: Variables analysed in this study were correlated to clinical outcomes. The age [median (range) years old] of women was 34 (22-44). Our results show women who were pregnant had a significantly (P<0.01) larger blastocyst width [median (range) µm] 184 (125-239) versus those who were not pregnant 160 (120-230). A significantly (P <0.01) larger [median (range) μm^2] blastocyst area 26099 (12101-45280) was seen in pregnant versus non pregnant women, 22251 (10992-37931).

A univariate logistic regression performed showed that blastocyst width [(OR = 1.026, 95% CI = (1.019, 1.033)] was found to be significant with P-value <0.0001. This shows that for every µm increase of blastocyst width, the odds of clinical pregnancy increase by 2.6%.

A univariate logistic regression performed also showed that blastocyst area [(OR = 1.00008, 95% CI = (1.00006, 1.00011)] was found to be significant with P-value <0.0001. The lower odds ratio is explained by a larger range in blastocyst area. This shows that, for every μm^2 increase of blastocyst area, the odds of clinical pregnancy increase by 0.008%. Hosmer-Lemeshow tests of calibrations were performed to verify calibration.
Limitations, reasons for caution: 6.6% of patients (44/664) were excluded from the study because dimensions could not be measured accurately. Although our findings show a clear effect of blastocyst dimensions on clinical pregnancy rate, further studies are necessary to confirm these observations.
Wider implications of the findings: Confirmation of our findings could result in establishment of a new algorithm to improve embryo selection at the blastocyst stage. However, this may not exclude the importance of other morphological features/grades in embryo selection.
Trial registration number: not applicabale

P-130 A prospective randomised control study comparing reproductive outcome of day 5 Quarter laser zona thinning assisted hatching (qLZT-AH) in frozen thawed embryo transfers

H. Sharma[1], K.D. Nayar[1], G. Kant[1], S. Draboo[1], M. Singh[1], R. Bhattachrya[1], S. Gupta[1], K.D. Nayar[1]

[1]Akanksha IVF Centre- Mata Chanan Devi Hospital, Reproductive Medicine, New Delhi, India

Study question: Can quarter zona laser thinning assisted hatching (qLZT-AH) improves reproductive outcome in day 5 frozen embryo transfer cycles?
Summary answer: Quarter laser thinning assisted hatching (qLZT-AH) on day 5 frozen thawed embryos is associated with improved implantation rate, clinical pregnancy rate over no assisted hatching.
What is known already: Hatching is a process where blastocyst escape the Zona pellucida (ZP) membrane prior to implantation. This is accomplished in-vivo by secretion of hatching factors and lysine production by trophectoderm of embryo but in in-vitro fertilisation when embryos are frozen under ultra low temperature, may lead to zona hardening. This may inhibit or reduce the chances of spontaneous hatching.

With the advent of laser assisted hatching (LAH), this complication could be overcome with focussed laser light to produce opening in ZP with a single pulse of few millisecond, with no mechanical, thermal or mutagenic side effects.
Study design, size, duration: A prospective randomised control study was conducted from 1st January to 31st December 2019. All patients whose frozen embryos were thawed on day 5 were included. Two hundred day 5 FET cycles were randomised by computer generated list and divided into 2 groups. Group A (n=100), in which Quarter laser zona thinning assisted hatching (qLZT-AH) was done after thawing while in group B (n=100) no laser assisted hatching was done after thawing.
Participants/materials, setting, methods: All normoresponder patients whose embryos were frozen on day 5 were included in this study and patients with endometrium pathologies were excluded. Quarter laser zona thinning assisted hatching (qLZT-AH) was performed after thawing in group A, where 25% of surface area and 50% thickness is removed by using laser while in group B no laser assisted hatching was done. Groups were compared on the basis of implantation rate, clinical pregnancy rate and miscarriage rate.
Main results and the role of chance: None of the Frozen embryo transfer cycle was cancelled and no loss of embryo was reported during thawing process. The cryosurvival rate was 96% in group A and 95% in group B which is in the range of cryopreservation key performance indicators. No significant difference in female age, BMI and AMH was observed between the two groups.
There was a statistically significant increase in implantation rate (35% vs. 23.07%, p=0.004) and clinical pregnancy rate (50% vs. 35%, p=0.031) when day 5 frozen thawed transfers assisted with qLZT-AH was done, while no difference in miscarriage rate (6% vs. 5.70%, p=0.906) was noted.
Limitations, reasons for caution: Larger randomised control studies are needed to strengthen these results.
Wider implications of the findings: We have demonstrated that day5 frozen embryo transfers assisted with quarter laser zona thinning assisted hatching (qLZT-AH) results in better reproductive outcome than if no hatching is done. This study strengthen the current trend of freezing more embryos at blastocyst stage and then assisting with qLZT-AH can further improve results.
Trial registration number: MCDH/2019/15

P-131 Is there a cohort-effect of low fertilization in human embryos? Analysis of live birth rate in 7,782 oocyte donation cycles.

M. Torra[1], D. Garcia[1], R. Vassena[1], A. Rodriguez[1]

[1]EUGIN, Clinica Eugin, Barcelona, Spain

Study question: Does fertilization rate *per se,* rather than having few embryos available for embryo transfer, affect live birth rates in ICSI cycles?
Summary answer: Fertilization rate *per se* does not affect clinical pregnancy and live birth rates after ICSI
What is known already: Low fertilization rates (FR) after ICSI reduce the embryos available for transfer. The number of embryos is a predictor of pregnancy and live birth (LB) rates, and controlling for this variable is imperative in order to understand the cohort effect of FR. A high percentage of non-fertilized oocytes at D+1 could underlie molecular and cellular alterations in either oocytes or sperm, which may partially affect also the embryos that continue their development. By comparing cycles with the same number of generated embryos, but different FR, we test whether FR *per se* affects pregnancy and LB rates after fresh embryo transfer.
Study design, size, duration: Retrospective cohort study of 7,782 oocyte donation cycles performed in one fertility clinic between 2011 and 2019. Between 5-14 metaphase II oocytes (MII) were assigned to each oocyte recipient. Each cycle gave rise to 4, 5 or 6 D+1 embryos after elective ICSI. Reproductive results were analyzed per groups with the same number of embryos (4-5-6), regardless of the number of MII needed to obtain those embryos (varying FR within groups).
Participants/materials, setting, methods: A minimum of 5 MII were assigned to each recipient hence, cycles with 4 embryos presented 33-80% FR, cycles with 5 embryos 38-100% FR, and cycles with 6 embryos 43-100% FR. For each group of embryos (4-5-6), the FR range was divided in categories containing >160 cycles each. Mean embryo morphological score, pregnancy and LB rates after fresh ET, were compared within groups and along FR available categories using ANOVA, Chi^2 and linear-by-linear tests.
Main results and the role of chance: Oocytes were inseminated with partner sperm in 84.1% of cycles (FR: 77.3%; LB rate: 35.6%) or donor sperm in 15.9% of cycles (FR: 77.9%; LB rate: 39.8%), equally distributed within the three groups. Fresh transfer of 1 (9.1% of cycles), 2 (90.7%) or 3 (0.2%) embryos was performed on D+2 (28.1%) or D+3 (71.9%) post-ICSI. Cycles with 4, 5 and 6 D+1 embryos were 2,873, 2,962 and 1,947, respectively. When considering all cycles that yielded 5 embryos, the most common occurrence, clinical pregnancy and LB rates were comparable across FR range (38.5%-100%), varying between 42.8% and 47.4% for clinical pregnancy (p=0.46), and between 35.7% and 39.9% for LB rate (p=0.56). Similarly, no significant differences were observed when considering mean embryo morphological score, pregnancy or LB rates in cycles that yielded 4 or 6 embryos (p>0.05 in all cases). Although clinical pregnancy rates and LB rates predictably increased with the number of embryos obtained overall (32.0%, 36.6% and 38.0%, for 4, 5 and 6 embryos respectively, p<0.001), we did not find an effect of FR *per se* on the performance of the cohort of embryos generated.
Limitations, reasons for caution: A minimum of 5 MII were assigned to recipients, therefore it was not possible to perform the analysis for the whole range of FR. Our results should not be extended to cycles with FR <30%. Studies specifically focusing on low and very low FR are needed to clarify this further.
Wider implications of the findings: FR *per se* does not affect reproductive outcomes. Cycles with lower FR after ICSI result in similar LB rate than cycles with higher FR if the number of generated embryos is the same. These results can inform patient counseling, and the management of oocyte donation programs.
Trial registration number: NA

P-132 Morphokinetic analysis of embryos originated from vitrified oocytes in infertile patients

A. Coello[1], M. Meseguer[1], M. Nohales[1], L. Alegre[1], J. Remohi[2], A. Cobo[1]

[1]IVIRMA Valencia, IVF laboratory, Valencia, Spain ;
[2]IVIRMA Valencia, Ob/Gyn, Valencia, Spain

Study question: Does oocyte vitrification alter embryo morphokinetic parameters in infertile patients?
Summary answer: Morphokinetics of embryos generated from vitrified oocytes is similar to that observed in fresh oocytes in infertile patients, except for time to first cell división.
What is known already: Although oocyte vitrification seems not to alter embryo morphology and clinical outcomes, some differences have been found in kinetic parameters of embryos originated from vitrified oocytes compared to those of embryos coming from fresh oocytes in donor cycles. These differences have revealed a delay of 1 hour in each cell division until early blastocyst stage in the vitrification group. Currently, there is lack of information about embryo morphokinetic parameters after oocyte vitrification in autologous cycles.
Study design, size, duration: This was a retrospective study including 2617 embryos originated from fresh oocytes (475 cycles) and 716 embryos derived from vitrified oocytes (118 cycles). All embryos were cultured in a time-lapse monitoring system (Embryoscope, Vitrolife and Geri, Genea). Embryo developmental events were annotated together with the corresponding timing of events in hours after intracytoplasmic sperm injection (ICSI).

Participants/materials, setting, methods: Kinetics parameters analyzed included: time to pronuclear appearance and fading (PNa, PNf), cleavage divisions until 8 cells (t2-t8), early, expanded and hatching blastocyst (tB, tBE, tBHi), as well as second cell cycle duration (cc2) and the synchrony in the division (s2). Embryos were classified according to the hierarchic tree model by Meseguer et al. 2011. Implantation and clinical pregnancy rates were also evaluated. The analyzed variables were compared using chi-square test and 95% confident intervals.
Main results and the role of chance: No significant differences in morphokinetic parameters were found between embryos originated from fresh and vitrified oocytes, except for t2. The proportions of embryos allocated to categories A–E in the hierarchical tree were similar between groups. The blastocyst formation rate was 52.0% (95% CI: 49.7%-54.3%) in the fresh oocytes group and 45.3% (95% CI: 40.7%-49.9%) in the vitrified oocyte group. No differences on implantation rate [41.5% (95% CI: 34.2%–48.8%) *vs.* 48.9% (95% CI: 34.9%–62.9%)] and clinical pregnancy rate [39.8% (95% CI: 31.6%-48.0%) *vs.* 55.0% (95% CI: 39.6%-70.4%)] were found between the fresh and vitrified group respectively.
Limitations, reasons for caution: The retrospective nature of this study is the main limitation. Additionally, the smaller sample size compared to previous morphokinetic analysis in donor cycles makes it difficult to drawn definitive conclusions.
Wider implications of the findings: The delay observed in donor cycles has not been confirmed in this study, most probably because patient's infertility condition is affecting the morphokinetics of embryos originated from fresh oocytes and, therefore, the effect of vitrification is not reflected in the analysis.
Trial registration number: 1511-VLC-062-AC

P-133 Amount of cell-free mitochondrial DNA in spent culture medium correlates with morphokinetic parameters of human blastocysts

M. Kobayashi[1], R. Suzuki[1], K. Tsukamoto[1], H. Hasegawa[1], T. Kyoya[1], A. Kikumoto[1], T. Sakurai[2], S. Saito[2], J. Kobayashi[2], H. Iwata[3]

[1]Kanagawa Ladies Clinic, Embryology Labolatory, Yokohama, Japan ; [2]Kanagawa Ladies Clinic, Department of Gynecology, Yokohama, Japan ; [3]Tokyo university of agriculture, Faculty of Agriculture Department of Animal Science, Atsugi, Japan

Study question: Does the amount of cell-free mitochondrial DNA (cf-mtDNA) in spent culture medium (SCM) correlate with the morphokinetic parameters (MPs) of human blastocysts?
Summary answer: The duration of blastocyst expansion (tEB - tB and tEB - tSB) and presence of blastocyst collapse correlated with the amount of cf-mtDNA in SCM.
What is known already: The mitochondrion is an important organelle for embryonic development. cf-mtDNA can be detected in SCM, and it has been reported that the amount of cf-mtDNA in SCM of early cleavage-stage embryos (day 3) reflects subsequent embryonic development and pregnancy outcomes. Accumulating evidence shows that MPs of human blastocysts obtained from time-lapse monitoring (TLM) facilitates the selection of competent blastocysts for clinical pregnancy. However, there have been no reports regarding the relationship between the amount of cf-mtDNA in SCM and MPs of embryos.
Study design, size, duration: This was a retrospective cohort study. We targeted 20 couples who underwent in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) treatment between January and December 2019. We measured the cf-mtDNA copy number in the SCM (20 μL) of embryos (total: 53) that were individually cultured to the expanded blastocyst stage for 5 or 6 days. Incubation was performed using a time-lapse incubator (EmbryoScope; Vitrolife, Sweden).
Participants/materials, setting, methods: Amount of cf-mtDNA, maternal age, type of insemination method, total embryo culture hours, morphological blastocyst quality (according to Gardner–Schoolcraft grading), certain MPs, and presence of blastocyst collapse (BC) were analyzed. DNA extracted from the SCM was quantified by real-time PCR (CFX Connect™; Bio-Rad, USA) using a specific primer set. Statistical analysis was performed using Spearman's correlation or Mann-Whitney-U-test.
Main results and the role of chance: The amount of cf-mtDNA did not correlate with maternal age ($r = -0.15$, $P = 0.28$), the total culture hours ($r = 0.18$, $P = 0.19$), or the type of insemination method (mean cf-mtDNA ± standard deviation; IVF: 12.0 ± 7.1, ICSI: 13.4 ± 7.8, $P = 0.56$). In addition, the mean cf-mtDNA content did not differ among morphological inner cell mass (A: 11.9 ± 8.0, B: 14.2 ± 6.8, C: 13.2 ± 5.0, $P = 0.31$) or trophectoderm (A: 12.1 ± 6.4, B: 12.1 ± 8.2, C: 15.9 ± 5.8, $P = 0.20$) grades. The analysis of MPs by TLM showed that the time taken from starting of blastulation to the expanded blastocyst (tEB - tSB) and from the blastocyst stage to the expanded blastocyst (tEB - tB) significantly correlated with the amount of cf-mtDNA in the corresponding SCM (tEB - tSB: $r = 0.46$, $P < 0.01$, tEB - tB: $r = 0.47$, $P < 0.01$). Furthermore, during the blastocyst stage, the mean cf-mtDNA content was higher for blastocysts that experienced BC than in those that did not experience BC (BC group: 14.9 ± 7.7 vs. non-BC group: 7.67 ± 3.9, $P < 0.01$).
Limitations, reasons for caution: The main limitation of this study was the small sample size; hence, we were unable to expand our hypothesis to clinical pregnancy outcomes at this point.
Wider implications of the findings: This is the first report showing an association between the amount of cf-mtDNA in SCM and MPs of blastocysts. It has been reported that the duration of expansion and presence of BC is associated with clinical outcomes. Thus, cf-mtDNA may be a useful non-invasive marker for blastocyst selection.
Trial registration number: not applicable

P-134 Original autologous partial oocyte vitrification program "ICSI-Vit-program": evaluation of the cumulative pregnancy rate and impact on the number of supernumerary cryopreserved embryos

P. Boyer[1], M. Gervoise-Boyer[1], C. Siraudin[1], A. Amar-Hoffet[1], D. Montjean[1]

[1]Hôpital Saint-Joseph, Laboratoire de Biologie de la Reproduction, Marseille Cedex 08, France

Study question: Does "ICSI-Vit-program" consisting in partial vitrification of MII-oocytes and delayed ICSI on thawed oocytes affect cumulative pregnancy rate after fresh and frozen embryo transfer ?
Summary answer: ICSI-Vit program during autologous ICSI cycle increases cumulative early pregnancy rate lowers the number of cryopreserved embryos and helps to promote single embryo transfer policy
What is known already: Partial vitrification of MII oocytes was introduced and validated in our laboratory as a tool to limit embryo freezing to meet legal requirements. Post-warming oocyte survival rate was reported to average 85% in IVF-treated patients. This implies a loss of 15% of oocytes and therefore a potential decrease in the number of embryos available. However, the top quality embryo rate is not higher than 50% with fresh oocytes and not all the supernumerary embryos can be cryopreserved. Data do not answer the question: could a non-survival oocyte after vitrification/thawing lead to a transferable embryo if it had been freshly fertilized
Study design, size, duration: To study cumulative pregnancy rate (the sum of pregnancies obtained in fresh oocytes, frozen embryos and when applicable vitrified/warmed oocytes cycles)
in couples included in ICSI-Vit program, we conducted a retrospective analysis of 378 ICSI-Vit cycles from September 2011 to December 2018 including 363 frozen embryo transfers compared to 420 conventional ICSI cycle without oocyte vitrification and fertilization of the entire MII oocyte cohort performed between January 2003 and July 2011including 353 frozen embryo transfers.
Participants/materials, setting, methods: Cycles with MII oocyte number ≥12 at pick-up were included, cycles without embryo transferred were not included. Data collection: Patient age, numbers of collected oocytes, vitrified oocyte after pick-up; fresh embryos obtained after fresh oocytes ICSI, Vit-fresh oocytes ICSI or frozen transferred embryos, early pregnancies (hCG blood test >100 IU) and deliveries were collected. Statistical analysis was performed. Cumulative rates were calculated including: fresh embryos transferred, frozen embryos and embryos from Vit-fresh oocytes.
Main results and the role of chance: We showed that vitrifying a part of Metaphase II oocytes during ICSI-Vit program allowed superior cumulative pregnancy rate when comparing with conventional ICSI (67,9%, *vs* 49,7% $p<0,05$). Besides, patients enrolled in ICSI-Vit program had fewer embryos transferred (1,2±0,5 vs 1,6±0,5 in conventional ICSI cycles [$p<0,05$]) and a mean of 1,5±0,7 embryo was transferred in vitrified/warmed oocytes cycles ([$p<0,05$]). Moreover we observed lower mean number of cryopreserved embryos in ICSI-Vit program during fresh embryo transfer cycles (1,2±1,4 vs 3,3±2,9 in

conventional ICSI cycles [p<0,05]) and 0,6±0,6 embryos were cryopreserved in vitrified/warmed oocytes cycles. Women included in both programs were of same mean age and had equal mean number of collected oocytes. However, fertilization rate of fresh oocyte was lower in ICSI-Vit program (65,7% vs 69,9% in conventional ICSI [p<0,05]), although no difference was found between fresh oocytes and sibling vitrified/warmed in ICSI-Vit program (65,8% vs 65,4%). These encouraging results may bring a tremendous change in IVF strategy by providing an opportunity for better oocyte use.

Limitations, reasons for caution: Oocyte vitrification can be proposed with oocyte storage instead of embryos when necessary. The compared groups were treated at different periods and cryopreservation methods were different (slow freezing in ICSI group and vitrification ICSI-Vit). This inconsistency may partially explain the benefit described. That point is being investigated.

Wider implications of the findings: Our results validate the vitrification of oocytes as an alternative to freezing embryos which may remain a real difficulty for various legal, ethical or religious reasons. ICSI-Vit program gives couples the opportunity to have multiple ICSI cycles with fresh embryo transfers in a context free of ovarian hyper-stimulation

Trial registration number: not applicable

P-135 Slow developing embryos undergoing compaction or cavitation on day 5 substantially contribute to live birth rates after single day 6 vitrified-warmed blastocyst transfer

J. Gunst[1], M. Vynck[2], A. Van de Vijver[3]

[1]AZ Sint Jan Brugge-Oostende, Laboratory Medicine - Centre for Reproductive Medicine, Brugge, Belgium ;
[2]AZ Sint Jan Brugge-Oostende, Laboratory Medicine, Brugge, Belgium ;
[3]AZ Sint Jan Brugge-Oostende, Centre for Reproductive Medicine, Brugge, Belgium

Study question: Does the status of slow developing embryos on day 5 and subsequent vitrification on day 6 impact clinical outcome in a vitrified-warmed transfer cycle?

Summary answer: Slow developing embryos that reach blastocyst stage by day 6 have a potential for live birth which is independent of the status on day 5.

What is known already: Transfer of a single blastocyst either in a fresh cycle or after vitrification / warming is becoming widely applied. An important aspect is the selection of the embryos that are eligible for transfer and/or for cryopreservation. Slow developing embryos on day 5, showing signs of compaction or cavitation, are either discarded or left in culture until day 6 for re-evaluation using scoring systems that assess blastocyst expansion and morphology of inner cell mass (ICM) and trophectoderm (TE). We investigated the potential of embryos that reached blastocyst grade >3 by day 6 in regard to clinical outcome after vitrification / warming.

Study design, size, duration: This single-center retrospective cohort study was undertaken between 2011/01 and 2018/12. Day 5 embryos with slow development, expressed as compaction or cavitation (blastocyst grade 1/2), were cultured to day 6. Blastocysts that had reached stage >3 were vitrified. We included 433 patients with 601 day 6 vitrified warmed blastocyst cycles resulting in 567 (94.3%) single vitrified warmed blastocyst transfers (SVBTs). Cycles with PGT, donor oocytes, re-vitrification and an aberrant day 5 status were excluded.

Participants/materials, setting, methods: Microdrop vitrification was performed using RapidVit Blast Kit/RapidVit Omni Kit (Vitrolife). Blastocysts were exposed to vitrification solutions 1&2 in microdroplets under oil, followed by solution 3 without oil and subsequent vitrification using a closed device (Rapid-i; Vitrolife). Warming was performed using RapidWarm kits, with no oil in Warm 1 solution followed by microdroplet warming in solutions 2, 3 and 4 under oil. All procedures were carried out at 37°C. Clinical outcome was assessed after SVBT.

Main results and the role of chance: For the 567 SVBTs that were performed on day 6, the status of development on day 5 was characterized as compaction (n=171; including embryos showing starting, partial and completed compaction; C), early blastocyst grade 1 (n=218; BL1) and blastocyst grade 2 (n=178; BL2). The percentage of day 6 SVBT with good quality blastocysts was 69.6%, 68.3% and 77.5% for status C, BL1 and BL2, and 30.4%, 31.7% and 22.5% for poor quality blastocysts, respectively. The overall resulting live birth rates (LBR) were 28.1% / 28.0% and 27.5% for day 6 SVBT from C, BL1 and BL2 derived embryos. LBR were higher for SVBT of good (BB or better) quality blastocysts (C: 29.4% / BL1: 30.2% / BL2: 31.9%) compared to those performed with poor (lower than BB) quality blastocysts (25.0% / 23.2% / 12.5%, respectively).

Considering the number of embryos included in this analysis, the LBR obtained from these slow developing embryos is unlikely to be a chance finding.

Limitations, reasons for caution: This is a single-center study, which was performed with a modified microdrop vitrification/warming protocol where all steps were carried out at 37°C and some steps did include an oil overlay. Thus, our results should be tested with a vitrification/warming protocol that uses room temperature.

Wider implications of the findings: Slow developing embryos on day 5 that reach blastocyst stage by day 6 substantially contribute to LBR in a vitrification/warming program. This holds even true for day 6 morphologically poor quality blastocysts. These findings, if confirmed, may challenge current practice to discard slow developing embryos on day 5 from vitrification.

Trial registration number: not applicable

P-136 Can monozygotic twinning in ART be explained by absence of zygotic polarization?

A. Mikich[1], G. Collar[1], M.I. Kulmann[2], M. Ferreira[3], S. Mattiello[3], N. Frantz[3]

[1]Federal University of Rio Grande do Sul, Morphological Sciences, Porto Alegre, Brazil ;
[2]Nilo Frantz Reproductive Medicine, Clinical embryology, Porto Alegre, Brazil ;
[3]Nilo Frantz Reproductive Medicine, Human Reproduction, Porto Alegre, Brazil

Study question: Is the absence of zygotic polarity associated with Monozygotic twinning (MZT) in ART cycles?

Summary answer: Yes, zygotic polarity was not observed in any of the zygotes that resulted in MZT. Enlarged periviteline space (PVS) and low Z-score were also detected.

What is known already: The incidence of MZT is increased after ART procedures. The origin of MZT associated with IVF is still unclear. Blastocyst culture and ICM splitting during hatching, ICSI and assisted hatching (AH) are some of the factors possibly associated with MZT. Little attention has been given to zygotic events that may contribute to monozygosity. Following sperm-oocyte interaction, pronuclei (PNs) develop and migrate toward a central position in the zygote. Alignment of PNs onto the polar axis is a fundamental event for normal first cleavage and development. Non-polarization leads to random ICM location in the blastocyst and anomalies in development.

Study design, size, duration: We retrospectively analyzed zygote images taken 16-17 hrs post-ICSI, from 14 frozen embryo transfer cycles that resulted in MZT gestations, between 2017 and 2019. The zygotes analyzed were the ones that gave rise to the blastocysts transferred in the 14 MZT gestations. Morphology of the transferred blastocysts and cycle characteristics were also examined.

Participants/materials, setting, methods: Observations of images and statistical analysis were carried out to investigate putative origin of MZT. The pattern of alignment of the PNs toward the polar bodies (PBs), angle between the PBs, periviteline space (PVS) and Z-score were assessed. Inner cell mass and expansion /hatching were assessed in day-5 blastocysts. Ovarian stimulation, oocyte number, fertilization rates, assisted hatching and cytogenetic analysis were considered. MZT was assessed by ultrasound at 6-7 and 8-9 weeks of gestation.

Main results and the role of chance: The transfer of a single blastocyst (7 cases) and of a single day-3 embryo (1 case) resulted in two GSs. One single blastocyst transfer resulted in three GSs, each with an embryo; another single transfer resulted in one GS with two embryos. When two blastocysts were transferred, two cases presented a single GS with two embryos and one case showed three GS with one embryo each. One double blastocyst transfer resulted in three GSs. 16 out of 18 zygotes did not present their PNs in alignment with the PBs. Two zygotes presented peripherally positioned PNs with their longitudinal axis aligned with one PB and the meridional axis aligned with the other PB. Majority of zygotes (15/18) presented a great angle separating the two PBs and an enlarged PVS (14/18). 12 zygotes scored as Z3-Z4 and 6 as Z2. ICM of eight blastocysts was large and tightly compacted, of seven embryos it formed two distinct cell masses. Blastocysts were expanded and 11 were herniating at transfer. There was no significant differences between the number of follicles, collected oocytes, fertilization rate, assisted hachting and cytogenetic analysis between the cycles with MZT and contemporary cycles that resulted in single gestation.

Limitations, reasons for caution: Results were based on observations made on zygotes and blastocysts of 14 IVF cycles. The occurrence of GSs derived from natural conception cannot be eliminated. A larger cohort of cases from different IVF centers should be studied to confirm our findings.

Wider implications of the findings: MZT represents an undesired IVF outcome, due to the possible adverse health consequences for the babies and their mothers. A careful zygotic assessment should be performed and taken into account, together with blastocyst ICM morphology, before elective single embryo transfer or double embryo transfer.
Trial registration number: N/A

P-137 The impact of single blastomere cell cycle duration of early cell division on blastocyst formation and blastocyst quality.

M. Kljajic[1], T. Krebs[2], D.H.A. Maas[2], N. Saymé[2]

[1]Saarland University Medical Center, IVF Laboratory, Homburg, Germany ; [2]Team Kinderwunsch Hannover, Department of Obstetrics- Gynecology & Assisted Reproduction, Hannover, Germany

Study question: Does the time duration of the second and third cell cycle for single blastomere affect blastocyst development and blastocyst quality?
Summary answer: The time duration of the second and third cell cycle strongly affects blastocyst development as well as blastocyst quality.
What is known already: In the last decade, the introduction of time-lapse technology enables almost continuous monitoring of embryo development. This technology generates comprehensive information regarding morphology and kinetics of embryo development and facilitates observation of dynamic, and often transient, events occurring between static observation periods. Together, these have been defined as 'morphokinetic' variables. Strong correlations between embryo kinetics and positive outcomes have been demonstrated in various studies. Some of the studies which involved cleavage time, cleavage interval, and cleavage synchrony reported that embryos with high developmental potential could be effectively identified using these parameters.
Study design, size, duration: Cell cycle duration is calculated using time-lapse, according to a single cell division. The injection time of ICSI was designated as "time zero" (t0), and computer software was used to calculate the time duration of the first (cc1a, t2-tPNf), second (cc2a, t3-t2; cc2b, t4-t2) and the third cell cycle (cc3d, t8-t4). Obtained results of cell cycle duration were later associated with the embryo capability to forming a blastocyst as well as with blastocyst quality.
Participants/materials, setting, methods: A total of 87 blastocysts from 20 patients undergoing an antagonist cycle for ICSI treatment between November 2019 and December 2019 were evaluated. All blastocysts were cultured in Embryoscope™ according to the manufacturer's specifications (Vitrolife, Sweden). The Gardner and Schoolcraft scoring system was used to describe blastocyst quality. Correlations between the data were calculated using logistic regression analysis. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using SAS software.
Main results and the role of chance: Morphokinetic data showed that the time duration of first (cc1a), and second (cc2a; cc2b) cycle were significantly different between embryos that reached the blastocyst stage and those embryos that did not. The first cell cycle was calculated as a time difference between pronuclei disappearance and the first cell cleavage, where time duration was significantly different ($p<0.05$) in successfully formed blastocysts versus arrested or non-blastulating embryos. What is more this parameter as well significantly affects blastocyst quality ($p<0.05$). Almost the same results were obtained for the second cell cycle where was confirmed that duration of single blastomere division (cc2a: $p< 0.001$) and (cc2b: $p<0.01$) significantly correlate with blastocyst formation potential. Therefore, blastocyst quality was also affected by the time duration of a single blastomere division cycle (cc2a, $p<0.01$; cc2b, $p< 0.05$). On the other hand, the duration of the third cell cycle (cc3d) between embryos which successfully reached the blastocyst stage and those embryos that failed were not statistically significant. What is more, blastocyst quality neither was affected by this morphokinetic parameter.
Limitations, reasons for caution: The disadvantage of the relatively small patient cohort was balanced with a wider range of patients' age (25-37). Further research should link these morphokinetic parameters with pregnancy rate and live birth rate as well.
Wider implications of the findings: The potential of the present findings is considerable as we believe that these results are helpful for a better understanding of the association between embryo morphokinetic parameters and blastocyst development. Also, the present work illustrates the possibility of additional information that can potentially be incorporated into an embryo classification model.
Trial registration number: 'not applicable'

P-138 Insights into early human development from abnormal fertilisation

G. Coticchio, Ph.D.[1], F. Gurioli[1], R. Sciajno[1], C. Lagalla[1], N. Tarozzi[1], M. Nadalini[1], C. Zacà[1], A. Borini[1]

[1]9.baby, Family and Fertility Center, Bologna, Italy

Study question: Does morphokinetic analysis of human abnormal fertilisation, one- (1PN) and three-pronuclear (3PN) fertilisation, offer cues to better understand the very early steps of development?
Summary answer: 1PN/3PN morphokinetic analysis gives insights into unknown aspects of fertilisation, concerning for example the role of the polar body, origin of pronuclei and pronuclear chromatin.
What is known already: The basic morphological attributes of human fertilisation have been known since the dawn of human IVF. For decades, such a knowledge has derived from static observation at a single time point. Initial application of Time-Lapse Microscopy (TLM) in the late 1990's revealed the beauty and morphokinetic complexity of fertilisation. However, in-depth dynamic observation of fertilisation has been pursued only in the last few years, with findings that have shed new light on cytoplasmic phenomena (e.g. the cytoplasmic wave and halo) and the importance of cell symmetry for embryo development. Unfortunately, abnormal fertilization has been largely neglected, despite potential for research.
Study design, size, duration: This retrospective study involved TLM observation of 378 normally (2PN) and abnormally (1PN and 3PN) fertilised oocytes utilised in ICSI cycles carried out between 2017 and 2019. Maximum three sibling fertilised oocytes (one normal and one or two abnormal) per patients were analysed to reduce possible patient-specific biases. Oocytes of patients with different diagnoses of infertility were included in the analysis, while cases involving cryopreserved gametes or surgically retrieved sperm were excluded.
Participants/materials, setting, methods: Microinjected oocytes were assessed by a combined TLM-culture system (Embryoscope). Oocytes not amenable to TLM assessment, due to excess of residual corona cells or inadequate orientation for observation of polar body II (PBII) emission, were not analysed. Fifteen parameters were identified and monitored, relevant to meiotic resumption, pronuclear dynamics, chromatin organization, cytoplasmic/cortical modifications and embryo quality. Diverse statistical tests were used depending on the numerical category of parameters compared between groups.
Main results and the role of chance: All major fertilisation phenomena – e.g. polar body II (PBII) extrusion, cytoplasmic wave, cytoplasmic halo presence and disappearance, PN migration, PN juxtaposition (where applicable) and nucleolar precursor bodies (NPB) redistribution – were discerned in the three PN categories. As a general trend, both 1PNs and 3PNs extruded the PBII with a delay of more than 0.6 hours ($P=0.0007$), but only 1PNs accumulated a developmental delay, starting from disappearance of the cytoplasmic halo, that amounted to almost 6 hours at the time of cleavage ($T=26.5$, 32.6 and 27.3 in 2PNs, 1PNs and 3PNs, respectively; $P<0.0001$). More intriguing finding, with significant developmental implications, derived from the observation of specific fertilisation events. For example: a) PBII extrusion was observed in the majority (45/75, 60%), but not all, of 1PNs and in a large proportion (61/136, 44.8%) of 3PNs; b) both the cytoplasmic wave and cytoplasmic halo were observed, not only in 2PN and 3PN, but also in all but one 1PNs; c) NPB clustering and localization in a confined nuclear region (NPB polarization) occurred in 2PNs (100%), 3PNs (96%) and, astonishingly, in most 1PNs (73%). Finally, on day 2/3, 1PNs were relatively more affected by developmental delay and blastomere fragmentation (data not shown).
Limitations, reasons for caution: This is a pilot study requiring extension and refinement of data, as well as independent verification.
Wider implications of the findings: These observations suggest a reappraisal of fertilisation. For example: 1PNs, with/without PBII emission, might be mono- or digynic, respectively; 3PNs not always derive by failed PBII extrusion; the cytoplasmic wave, generated by the microtubular aster, can form in the absence of the male pronucleus; NPBs polarize in isolated pronuclei.
Trial registration number: Not applicable

P-139 The impact of Vitrification in the outcome of IVF in an oocyte donation program.

P. Tatsi[1], T. Chartomatsidou[1], E.G. Papanikolaou[2], R. Najdecki[2], F. Chouliara[2], I. Asuchidou[2], C. Ioakeimidou[2], E. Timotheou[1]

[1]Assisting Nature, Embryology Laboratory, Thessaloniki, Greece ; [2]Assisting Nature, Clinical Department, Thessaloniki, Greece

Study question: How does Vitrification and double Vitrification (cryopreserved embryos produced from cryopreserved oocytes) affect the result of an oocyte donation program?
Summary answer: Oocyte and embryo Vitrification does not affect the outcome of IVF with donor oocytes. However, cryopreserved embryos from vitrified donor oocytes exhibit reduced implantation potential.
What is known already: Vitrification of oocytes, embryos and blastocysts is successfully performed worldwide in IVF Treatments and Oocyte Donation Programs. Vitrification of Oocytes and Embryos does not impair oocyte and embryo viability or implantation potential. No statistically significant difference is observed in the outcome after blastocyst embryotransfer originated from fresh versus vitrified donor oocytes. In addition, embryos derived from fresh donor oocytes show an extremely high implantation potential and exceptional clinical pregnancy and live birth rates. On the other hand, few is known regarding cryopreserved embryos that were produced from cryopreserved donor oocytes.
Study design, size, duration: This study was conducted between January 2015 and December 2018 to examine the impact of Vitrification and compare the pregnancy and live birth rate after embryotransfer of day 5 embryos of fresh oocytes-fresh embryotransfer (FROFRE) versus vitrified donor oocytes-embryotransfer (CROFRE) and cryopreserved embryos derived from fresh oocytes (FROCRE) and cryopreserved embryos originated from cryopreserved oocytes (CROCRE). A total of 425 donations were included in the study.
Participants/materials, setting, methods: A total of 425 oocyte recipient cycles were included in the study. 111 FROFRE cycles, 92 CROFRE cycles, 184 FROCRE and 38 CROCRE cycles. 896 fresh oocytes were utilized in FROFRE cycles and 736 vitrified oocytes were thawed and used in CROFRE cycles. ICSI was implemented in all cases and embryotransfer and vitrification was performed on day 5 on blastocyst stage. Positive hCG, clinical pregnancy and live birth rates were monitored.
Main results and the role of chance: Positive β-hCG, clinical pregnancy rate and live-birth rate per embryotransfer were compared between our groups, FROFRE-CROFRE-FROCRE-CROCRE. Furthermore, embryo quality and blastulation rate was similar among fresh donated oocytes and from vitrified oocytes. β-hCG was 69%, 59% and 54% between FROFRE, FROCRE and CROFRE groups respectively. No statistically significant difference was observed in the clinical pregnancy rate, which was 55% (FROFRE), 52%(FROCRE) and 47%(CROFRE) and the live birth rate 46% (FROFRE), 44% (FROCRE) 42% (CROFRE). The CROCRE cycles exhibited the lowest rates 35% (β-hCG), 19% (clinical pregnancy) and 15% (live birth). Oocyte vitrification and warming is common and effective method of delivering donor oocytes to patients and success rates all over the world are as high and similar to results in fresh donor egg cycles. One remaining question is if these previously frozen oocytes, once warmed, inseminated and cultured to later embryonic stages can be again re-vitrified and warmed with similar results as in fresh oocyte donation cycles. According to our findings there is a significant impact of double-vitrification in pregnancy and live birth rates.
Limitations, reasons for caution: Although our primary results show that clinical impact in success rates of double-vitrification is significantly impaired in our oocyte donation program, this needs further investigation in larger randomized studies. Few CROCRE cases were included and the embryos studied were chosen for second, third or final embryotransfer after previous CROFRE cycle.
Wider implications of the findings: It is important to study and perceive if embryos derived from vitrified donor oocytes perform similarly to fresh oocytes after double vitrification and warming. The couples interested in oocyte donation and IVF treatments should be thoroughly informed about their options and success rates.
Trial registration number: Not Applicable

P-140 Clinical pregnancy and perinatal outcomes of twice-frozen-thawed embryo transfers: a retrospective comparative study

B. Cai[1], W. Yali[1], X. Yanwen[1], Z. Canquan[1]

[1]First Affiliated Hospital of SunYat-sen University, reproductive medicine center, Guangzhou-Guangdong, China

Study question: Dose repeated-frozen-thawed human embryos can achieve comparable clinical pregnancy and perinatal outcomes to conventional once frozen-thawed embryos ?
Summary answer: Acceptable clinical outcomes could be expected from transfer of recryopreserved blastocysts, while perinatal outcome was not affected and clinical pregnancy parameter was lower but not significant.
What is known already: Vitrification is becoming the most widely adopted embryo cryopreservation technique for higher embryo survival rate and live birth rate than other methods.In order to avoid the risk of multiple pregnancies, most countries recommend reproductive centers to transfer only one blastocyst per cycle, causing surplus of surviving embryos might occasionally be used in FET cycles. But to our knowledge, limited data was available for FET outcome with twice cryopreserved human embryos, and the sample size was small .With the increase number of repeated cryopreserved embryo transfer cycles, the impact of repeated-frozen-thawed manipulate to clinical pregnancy outcomes needs further to be elucidated.
Study design, size, duration: The purpose of this retrospective cohort study was to evaluate the pregnancy and perinatal outcome of revitrified human day5/6 blastocysts derived from surplus of surviving blastocysts in the last FET cycles. Cohorts of 320 transfered blastocysts with either twice-frozen-thawed and once-frozen-thawed between January 2016 and December 2018 were included in the study. Only IVF/ICSI cycles were included,PGT cycles were excluded. Match-pair analysis was used to control for some patient basic characteristics,then compared two groups.
Participants/materials, setting, methods: During the match-pair analysis, we paired twice-frozen-thawed and once-frozen-thawed groups' patients data through maternal Age,body mass index, endometrial thickness. The two groups' paired ratio was 1 (80 cycles):3 (240 cycles). The total 320 transfered blastocysts gave live birth to 105 infants, of which twice-frozen-thawed (22 infants) and once-frozen-thawed groups (83 infants). Finally,we compared clinical pregnancy outcome parameters between the two groups,included implantation,clinical pregnancies,live deliveries, miscarriage rate, infants' birth body length, birth weight and so on.
Main results and the role of chance: We regarded once-frozen-thawed FET as the control group to compare with twice-frozen-thawed FET group. By statistical comparison(twice-frozen-thawed versus control),we found maternal age(31.60±4.24 versus 32.33± 4.34, P >0.05,), body mass index (21.62±3.34 versus 22.53± 4.74kg/m², P >0.05), endometrial thickness (9.78±1.54 versus 9.53± 1.64, P >0.05), Embryo transfer cycles(2.11±1.34 versus 2.83± 1.55, P >0.05) and number of embryos transferred (1.0 versus 1.0, P >0.05) were all not significantly different between two groups. While clinical pregnancy rate(36.25% versus 46.25%,P >0.05) , implantation rate (36.25% versus 46.25%, P >0.05) and live birth rate (27.50% versus 34.58%, P >0.05) were all lower in twice-frozen-thawed group,but the difference was not significant by the Chi-square test .Unexpectedly , miscarriage rate (7/29-24.14% versus 29/111-26,13%,P >0.05) was lower in twice-frozen-thawed group but not significantly. As for the perinatal data, no significantly differences were observed in gestational age(38 w±3.4d versus 38 w±5.3d, P >0.05), sex ratio(11/11 versus 48/35,Boy/Girl), birth body length (49.15±4.12cm versus 49.09±3.88 cm, P>0.05) and birthweights (3220.00±422.3 g versus 3116.99±453.6, P>0.05) between groups. Likewise, in the live birth individuals,there were no significant differences between the two groups on the incidence of pre-term birth (3/22 versus 11/83), low birthweight(2/22 versus 7/83), small for gestational age, large for gestational age and macrosomia.
Limitations, reasons for caution: Because the refrozen group's sample size was limited,we did not distinguish some confounding factors associated with clinical pregnancy outcomes, included type of infertility,cause of infertility, different development stage,and the embryo morphological score,insemination method,endometrial preparation protocol , embryo cryopreservation duration and the long-term effects of re-vitrification method on offspring's health outcomes.
Wider implications of the findings: The results provide feasibility to use refrozen blastocysts in FET which can obtain an acceptable clinical outcome expectation ,when there is no other primary cryopreservation blastocyst to choose. Meanwhile, patients should be fully informed of possible reduction in clinical outcome parameters.
Trial registration number: not applicable

P-141 Comparison of clinical results of embryo culturing using last generation incubators: is there a better option?

M.D.L.Á. Valera Cerdá[1], C. García Gimeno[1], L. Bori Arnal[1], J.M. De los Santos Molina[2], A. Pellicer Martínez[3], M. Meseguer Escrivá[2]

[1]IVIRMA, Research Laboratory, Valencia, Spain ;
[2]IVIRMA, IVF Laboratory, Valencia, Spain ;
[3]IVIRMA, President- Reproductive Medicine, Valencia, Spain

Study question: Is there a significant difference in clinical results using different last generation time-lapse incubators (TLM) for embryo culturing, using benchtop incubators (CI) as a reference?
Summary answer: We did not find significant differences in pregnancy and implantation rates between last generation incubator systems. When grouped, TLM showed better outcome compared to CI.
What is known already: Time-lapse systems (TLM) bring to IVF laboratories a clear advantage, providing new information of embryo development without perturbing their culture. However, their performance as incubators must be tested versus those conventional benchtop incubators (CI), to assure they provide a proper environment for embryo culturing. To date, there is insufficient evidence of actual significant differences in clinical outcomes varying on the type of incubator used, although some studies show a slight improve with time-lapse systems. Previously published studies have found a significant increase in good-quality blastocyst and euploidy rates using time-lapse incubators. However, comparative studies on the matter are usually incomplete.
Study design, size, duration: We present a retrospective study including 8446 ICSI cycles performed in IVIRMA Valencia from two consecutive years, both with own and donated oocytes. Fertilized oocytes were cultured until blastocyst stage using different systems: CI (Astec, Japan) or incubators with TLM, including Embryoscope, Embryoscope Plus (Vitrolife, Denmark) and Geri (Genea, Australia). Out of 26100 viable embryos, 9817 were selected for transfer, 7574 in single embryo transfer (SET), with clinical outcome data of 9103 transferred embryos available.
Participants/materials, setting, methods: All incubators included in the study are well stablished and routinely used in the clinic for embryo culturing. Scoring and selection for transfer or freezing were performed according to the ASEBIR criteria, by morphological assessment combined with morphokinetic selection methods. A statistical analysis was performed for comparing clinical results between incubators, using the Statistical Package for Social Sciences (SPSS). Results are presented as pregnancy, ongoing pregnancy and implantation rates per incubator, separately by oocyte origin.
Main results and the role of chance: The statistical analysis shows that there is no significant difference in the clinical results of cycles using the different types of TLM. For autologous cycles, the pregnancy rate of fresh transferred embryos (N=744) was 59.4% for Embryoscope (ESD), 64.1% for Embryoscope Plus (ESD+) and 61.7% for GERI (P=0.305). For Egg donation cycles, having a total of 2384 transferred embryos, pregnancy rates resulted in 72.9% for ESD, 74.1% for ESD+ and 69.3% for GERI (P=0.173). Regarding implantation rates, in procedures with own oocytes resulted in 48.14% using ESD, 50.35% for ESD+ and 50.79% using GERI incubators (P=0.855). As results for cycles with donated oocytes, the implantation rate was 61.42% for ESD incubators, 61.99% for ESD+ and 58.78% with GERI, not having a statistically significant difference (P=0.288). When comparing CI with TLM for autologous cycles, the pregnancy rate of fresh transferred embryos was 69.0% for TLM and 66.1% for CI (P=0.046), while ongoing pregnancy also resulted significantly higher in the standard cycles when compared TLM 60.9% vs CI 54.5% (p=0.047).
Limitations, reasons for caution: Other variables should be assessed as well, such as embryo quality, good-quality embryo rate and life-birth rate per incubator. Furthermore, this study only compares four types of incubator but there are many more available.
Wider implications of the findings: The results of this study can serve as a quality check of the procedures in the clinic. It reassures that all TLM used in the clinic yield similar results and there is no bias in the clinical results depending on which TLM is used.
Trial registration number: Not applicable

P-142 Increased DNA fragmentation in blastocysts triggers the release of miR-294 in spent culture medium

D. Makri[1], W. Maalouf[1]

[1]University of Nottingham, Child Health- Obstetrics- and Gynaecology, Nottingham, United Kingdom

Study question: To study whether extended apoptosis in embryonic blastomeres enhance the packaging and release of miR-294 by blastocysts.
Summary answer: Induced DNA fragmentation at the early blastocyst stage causes increased release of miR-294 in the culture medium.
What is known already: MicroRNAs are important regulators of cell signaling, growth, and cell death. Pre-implantation embryos release microRNAs in the surrounding micro-environment and cell-free microRNAs reflect on the quality and ploidy status of embryos. It is known that miR-290 members are regulators of apoptosis in cells. In pre-implantation embryos, miR-294 levels in media directly correlate with apoptosis in the blastomeres. However, there is a lack of mechanistic explanation behind this observation. It is not clear whether high miR-294 in cells causes DNA fragmentation or increased apoptosis results in intense release of miR-294, which could serve a secretory function.
Study design, size, duration: Mouse zygotes (N=28) were cultured to the small blastocyst stage. At this stage, UV radiation was used to induce DNA damage in order to mimic the final stages of apoptosis. The culture was extended to allow for cellular responses to DNA damage. The blastocysts were stained at Day 5 of development to verify DNA damage. Media samples were individually processed and analysed for miR-294. UV-treated and control samples were compared for microRNA levels (t-test).
Participants/materials, setting, methods: Frozen mouse zygotes (B6C3F-1 x B6D2F-1) were cultured at 37°C, 5% CO_2 to the early blastocyst stage. The blastocysts (N=15) were exposed to UV radiation (5 s, 302 nm) and cultured for 16 more hours. Control embryos were cultured in parallel (N = 13). The embryos were stained with the TUNEL method to assess DNA damage. Media samples (20µl) were individually analysed for miR-294 expression using PCR technology.
Main results and the role of chance: UV radiation can be used to induce DNA damage and study apoptosis-triggered mechanisms. For embryos, exposure to 302 nm for 5 s at the early blastocyst stage causes increased DNA breakage but maintains blastocyst viability for enough time to investigate molecular changes. The direct effects of UV radiation on DNA breakage was verified, with the UV-treated group showing higher extent of apoptosis (and reduced cell count) compared to the control embryos. The average miR-294 Ct in the SCM of the UV group was 25.1 ± 1.1 (ΔCt 6.3 ± 1) and in the control group 27.1 ± 1.5 (ΔCt 8.3 ± 1.5). The results from the t-test showed that the blastocysts with UV-induced DNA damage released significantly more miR-294 in the media compared to controls ($p<0.01$).
Limitations, reasons for caution: These results are limited to mouse embryos and further research is needed for applications in other species.
Wider implications of the findings: UV radiation is a useful approach to mimic the last stages of apoptosis and study related cellular responses. Apoptosis in pre-implantation embryos triggers the intense packaging and release of miR-294 in the extra-cellular environment with possible actions as a singaling molecule for communication with the endometrium.
Trial registration number: Not applicable

P-143 Oocyte central granularity is associated with reduced fertilization rates and altered fertilization dynamics

C. Moutier[1], M.C. Guglielmo[1], P.V. Novara[1], F. Brambillasca[1], A. Bartolacci[1], M. Mignini Renzini[1], M. Dal Canto[1], J. Buratini[1]

[1]Biogenesi Reproductive Medicine Centre, Istituti Clinici Zucchi, Monza, Italy

Study question: Is cytoplasmic central granularity (CCG) in oocytes associated with alterations in fertilization rates and dynamics?
Summary answer: Oocyte CCG is associated with reduced fertilization rates, as well as delayed pronuclei disappearance and first cell division after ICSI.
What is known already: Oocyte quality is a major determinant of ICSI success. Oocytes are routinely selected according to morphological criteria before ICSI, but the relationship between different oocyte morphological features and developmental competence is not entirely known. Oocyte CCG is associated with alterations in the actin cytoskeleton and meiotic spindle, both playing critical roles in meiosis completion, pronuclei formation and all steps of embryo mitotic divisions. Faster fertilization morphokinetics is associated with higher rates of embryonic development.
Study design, size, duration: We performed a retrospective analysis comparing fertilization rates and fertilization morphokinetic parameters of control (morphologically normal) and CCG oocytes, obtained from patients under 40 years of age subjected to ICSI in our Fertility Centre from January 2016 to July 2019. ICSI cycles were selected in order to equally distribute infertility factors in both experimental groups. Morphokinetic parameters were assessed by time-lapse technology.
Participants/materials, setting, methods: A total of 1075 metaphase II oocytes were injected by ICSI obtaining an overall fertilization rate of 76,5% (823/1075). Morphokinetic parameters until the first division were compared

between CCG and control oocytes (n=141/group). Maternal age and body mass index (BMI) did not differ between experimental groups (CGC: age=34,44 ± 3,13; BMI=23,35 ± 4,13. Control: age=34,40 ± 3,14; BMI=22,64 ± 3,42).
Main results and the role of chance: Fertilization rate was significantly lower in CCG oocytes compared to control [69,1% (141/204) *vs.* 78,3% (682/871); p < 0,01]. Time of pronuclei fading was longer (p = 0,02) for CGC oocytes compared to control oocytes (24,52h ± 3,39 and 23,72h ± 4,13 respectively). The first mitotic division was delayed in embryos obtained from CCG oocytes compared to control embryos (27,43h ± 3,57 *vs.* 26,53h ± 3,88 respectively, p = 0,02).
Limitations, reasons for caution: The retrospective nature of the study and the need of further studies to assess if CCG is also associated with subsequent embryo competence to implant and provide a live birth.
Wider implications of the findings: Our study contributes to a better understanding of the relationship between oocyte morphology and quality, while providing valuable references for the improvement of ICSI outcomes.
Trial registration number: Not Applicable

P-144 A metabolomics approach to identify aneuploid embryos to increase the effectiveness of ART cycles

S. Cabello-Pinedo[1], H. Abdulla[2], M.L. Seth-Smith[1], M. Escriba[3], J. Crespo[3], S. Munné[1], J.A. Horcajadas Almansa[1]

[1]Overture Life SL, Scientific department, Madrid, Spain ;
[2]Texas A&M University Corpus Christi, Chemistry, Corpus Christi, U.S.A. ;
[3]Juana Crespo Clinic, Embryology, Valencia, Spain

Study question: Can euploid embryos be identified in a non-invasive manner by measuring the concentration of specific metabolomic biomarkers in spent culture media?
Summary answer: Specific metabolites in embryo spent culture media are correlated with euploidy status of blastocyst stage embryos.
What is known already: Embryo Implantation potential and euploidy rates decrease with advancing maternal age. Preimplantation Genetic Testing (PGT) has been used to avoid the transfer of aneuploid embryos, which would not result in a successful pregnancy. However, embryo biopsy is invasive and operator-dependent resulting in wide variability in pregnancy rates. Although DNA can be detected in spent media, correlation with trophectoderm biopsy varies widely between different studies. Recently, metabolomics studies on spent culture media have shown promising results to predict implantation potential. In this study we aim to determine if euploidy status can also be ascertained by non-invasive metabolomics analysis.
Study design, size, duration: This study includes spent media samples collected before trophectoderm biopsy from embryos that were later analyzed by PGT (NGS). Among them, 60 out of 116 were classified as aneuploid, 42 as euploid and 14 as mosaic. Samples were analyzed to find metabolites differentially abundant in the different embryo groups, leading to the definition of a method that allows selection of euploid embryos for transfer without the need for biopsy avoiding possible damage to viable embryos.
Participants/materials, setting, methods: Patients undergoing PGT were included in this study. Spent media samples were collected just before biopsy, ultrafiltered to remove molecules >3KDa and run through a UPLC- Fusion Orbitrap MS/MS system at 500,000 FWHM mass resolution. Different machine learning techniques were applied to reduce the huge number of metabolites to a limited number of informative biomarkers for euploidy.
Main results and the role of chance: The analysis of mass spectrometry (MS) led to the identification of a sub-set of biomarkers whose concentration differed between aneuploid, euploid and mosaic embryos. The concentration of this subset of biomarkers was increased in aneuploid embryos and reduced in euploid and mosaic embryos. Fifty-four out of the 60 aneuploid embryos (90%) showed a reduced concentration of aneuploid profile specific metabolites and different from euploidy.

The challenge of this study relies on the wide range aneuploidy profiles found in blastocysts, from monosomies to trisomies for each possible chromosome, alone or in combination with other abnormalities. Each aneuploidy may produce different metabolic alterations, resulting in very diverse and heterogenous metabolic profiles. Given the diversity of the aneuploid group, and the positive identification of such profiles into the aneuploid category, the sub-set of identified biomarkers are probably definitory of a particular\specific metabolic profile and could hardly be found by chance.
Limitations, reasons for caution: The study was retrospective but a prospective clinical trial is underway. In addition, all the samples were collected in the same clinic using the same culture media. The test needs to be validated in multiple clinics and culture media.
Wider implications of the findings: 90% aneuploidy positive identification without biopsy is as high as the highest reported NI-PGT results. Further advantages of metabolomics is that is cheaper than NI-PGT (NGS) and does not require change in embryology protocols. Combined with markers for embryo viability other than euploidy might yield further improvements in embryo selection.
Trial registration number: NA

P-145 A new role of Repulsive Guidance Molecule C as a ligand of neogenin enhanced in follicular development of ovary in the mouse

J.H. Lee, Ph.D.[1,2], Y.J. Kim[1], K.H. Choi[3], K.Y. Kang[3], H.S. Kim[3], H. Jeong[3], H. Kang[4], Y.R. Park[3], S.J. Kim[5], M.K. Kwon[5], H.O. Kim[5], M.K. Koong[5], Y.S. Kim[5], T.K. Yoon[5], J.J. Ko[2]

[1]CHA Fertility Center Seoul Station, Reproductive Molecular Medicine, Seoul, Korea- South ;
[2]CHA University, Biomedical Science, Seoul, Korea- South ;
[3]CHA Fertility Center Seoul Station, Embryology Lab, Seoul, Korea- South ;
[4]CHA Fertility Center Seoul Station, Andrology Lab, Seoul, Korea- South ;
[5]CHA Fertility Center Seoul Station, IVF Clinic, Seoul, Korea- South

Study question: In the ovary, Repulsive Guidance Molecule c (RGMc) stimulated neogenin signal has a specific function for follicle development and oocyte maturation?
Summary answer: Neogenin is specifically localized at the follicle and RGMc as ligand promotes follicle development and the maturation of the oocyte in the ovary.
What is known already: Neogenin has reported a gene level expression in the mammalian ovary. However, neogenin as a multiple function receptor remains unknown as the main role and ligand for folliculogenesis in the ovary of mammals.
Study design, size, duration: This is an animal model study for the role of and RGMc as a ligand of neogenin during the follicular development phase in the ovary of the mouse. We compared the study between controls (n=25 head) versus RGMc treatment (n=25 head) groups. We analyzed the follicular development ratios of mouse ovary with RGMc + PMSG induction. And we study uP- and down-regulation whole transcriptome clusters of RGMc treated ovary.
Participants/materials, setting, methods: We identified the gene, the protein expression of neogenin and the localization in the ovary. Then we studied the role of the neogenin through superovulation by PMSG with RGMc treatment as a ligand of neogenin for *in vivo* follicle development activation. And afterwards, we performed an entire transcriptome next generation sequencing process for the identification of the RGMc effect, which linked the specific factors to the follicle development in the ovary.
Main results and the role of chance: Neogenin is abundantly expressed in the ovary and located at the developmental oocyte phase from primordial and antral follicles. Therefore, the RGMc treated ovary exhibits a 40% increase in the number of follicles compared with the control ovary. And RGMc treated ovary present Oct3/4, Nanog, p63, and Neogenin significantly higher gene expressed than control ovary. The especially experimental group, Nanog and p63 three times higher expression than the control group. In the western blot data also present similar pattern like RT-PCR. To identify the transcriptomic signature between RGMc-treated and control mice, RNA-seq was performed. As a result, total of 275 genes was differed by more than 4-fold change between the RGMc-treated and control groups. Among the total 275 genes, 197 and 78 genes were relatively uP-regulated in RGMc-treated and control group, respectively (Figure 4A and 4B). The 197 genes overexpressed in the RGMc group were significantly enriched to cilium organization, axonemal dynamin complex assembly, microtubule bundle formation, icosanoid metabolic process, prostaglandin metabolic process, and prostanoid metabolic process. The 78 genes down-regulated in the RGMc group were significantly enriched to ovulation cycle process, hormone biosynthetic process. Specifically, RGMc treated ovary shows specific uP-regulation factors as a prostaglandin linked signal.
Limitations, reasons for caution: This study did not the analysis of the human samples because of animal experiments for follicle development and oogenesis. Therefore, further investigations aimed specific analysis of RGMc in human at improving controlled ovarian hyperstimulation study with RGMc of poor ovarian response.

Wider implications of the findings: RGMc as a ligand of neogenin involved follicle development and maturation of oocytes. RGMc stimulated the PSDN signaling pathway for follicular development. This platform can apply to poor ovarian responses as a specific stimulator for enhanced follicle development for the *in vitro* fertilization patients of the poor ovarian responder.
Trial registration number: 2018R1D1A1B07050138, 2018R1C1B5045516, 2018R1D1A1B07044016 and 2019R1A2C1086882.

P-146 Soluble protein Cripto-1 promotes aberrant cell divisions in human embryonic cells

V. Porokh[1], P. Vanhara[1], Z. Holubcova[2], A. Hampl[1]

[1]Masaryk University- Faculty of Medicine, Histology and Embryology, Brno, Czech Republic ;
[2]Reprofit International- Clinic of Reproductive Medicine, Research&Development, Brno, Czech Republic

Study question: Do secretory factors present in culture media affect the fidelity of mitotic divisions during preimplantation embryo development?
Summary answer: Cripto-1 signaling induces the formation of multipolar mitoses hence increasing the risk of aneuploidy in early human embryogenesis.
What is known already: Mitotic errors are common in preimplantation development. Abnormal segregation of genetic material results in aneuploidies, spontaneous abortions, and birth defects. Despite its major importance for assisted reproduction, our knowledge of molecular mechanisms governing cell division in early human embryos remains rudimentary.
Study design, size, duration: A basic research study involving low-passaged human embryonic stem cells (hESCs; lines CCTL12, CCTL14, MUES1) and spent cultivation media from 30 IVF cycles. Media from successful cultures with >80% fertilized eggs developing into blastocysts (n=15) were compared with cultivations in which all embryos arrested in cleavage stage (n=15). A combination of microscopic and immunoanalytical assays was used to investigate the link between embryonic secretome and cell division errors.
Participants/materials, setting, methods: The pluripotent stem cells were derived from the inner cell mass of human blastocysts donated for research. The single-step medium (CSCM-C, Irvine) from each group culture was collected on the day-5/day-6 following ICSI and frozen in -20°C prior to analysis. The immunoaffinity proteomic array and ELISA were performed to assess soluble factors in the stem cell/human embryo microenvironment.
Main results and the role of chance: Immunofluorescent staining of cultured hESCs revealed that 9-15% of mitotic cells exhibited supernumerary centrosomes and aberrant mitotic spindles. The presence of more than 2 centrosomes and the formation of multipolar division apparatus was found to promote the incidence of aneuploidy in later stem cell generations. Immunoaffinity assay of 120 soluble proteins detected 18 molecules that were specifically produced by low passage hESCs with a high incidence of multipolar mitoses. Functional experiments led to the identification of the soluble form of Cripto-1protein as a factor capable to induce multicentrosomal phenotype. To complement results from the hESCs model, the analysis was undertaken on the spent media from human embryo cultures. Our finding that the soluble Cripto-1 was enriched in the microenvironment of human embryos which failed to reach the blastocyst stage supports the notion that this secretory molecule adversely affects genomic stability during preimplantation development.
Limitations, reasons for caution: The limitation of this preliminary study must be interpreted with caution due to the small study size. Further research is needed to evaluate whether the secretion of Cripto can be used as a negative indicator of embryo´s developmental competence in clinical practice.
Wider implications of the findings: Here, we present soluble Cripto-1 as a potential inducer of multipolar mitosis that may compromise the developmental potential of human embryos. The future investigation of soluble factors in the spent embryo culture medium holds the potential to discover novel non-invasive biomarkers of human embryo quality and developmental potential.
Trial registration number: not applicable

P-147 Effect of Hazardous Air quality index on embryo development in an IVF laboratory in New Delhi, India

G. Kant[1], K.D. Nayar[1], M. Singh[1], S. Draboo[1], H. Sharma[1], F. Khan[1], S. Gupta[1], R. Bhattacharya[1], D. Nayar[1]

[1]Akanksha IVF Centre, Reproductive Medicine, New Delhi, India

Study question: Does the hazardous atmospheric air can impact embryo development in the IVF laboratory even after standard air quality management and use of air filters?
Summary answer: There was a decrease in key performance indicators of IVF lab with increased fragmentation, poor embryo development and reduced reproductive outcome.
What is known already: According to WHO survey amongst 1650 cities in the world, Delhi the capital has worst air quality. With the air quality index falling drastically from Moderate (101-200) level between January to September to severe or hazardous (500+) level from October to December. The factors for poor air quality is stubble burning, road dust, cold weather and vehicle pollution. Studies have supported that air quality is critical to embryo development and for overall success of IVF. Both animal and human studies have suggested an association between poor air quality conditions and impaired embryo development, resulting in decreased implantation and pregnancy rates.
Study design, size, duration: A retrospective study was conducted from 1st January to 31st December 2019. Patients were divided in 2 groups. Group (A) from October to December, when atmospheric air quality was hazardous and Group (B) patients from January to September, when atmospheric air quality was within normal range. Both groups were compared on the basis of fertilisation rate, fragmentation rate, Day 3 grade A embryo development rate, implantation rate and clinical pregnancy rate.
Participants/materials, setting, methods: All patients undergoing fresh day 3 embryo transfers from the month of January to December were included. Out of the total 276 patients, 60 patients had their embryo transfer in the month of October to December (study group), while 216 were those from month of January to September (control group). Same culture media was used throughout the period. There was no change in clinical or embryology team.
Main results and the role of chance: The average AQI in Delhi was recorded around 500 between October to December 2019, while the maximum was recorded more than 1200. The quality of atmospheric air was correlating with the quality of embryo development. In group A (Oct-Dec). Fragmentation rate was significantly higher in Group A than Group B.(Fragmentation <10% : 53.03% vs 70.6%; p= 0.00001, Fragmentation 10-20% :30.80% vs. 18.78%; p=0.0002, Fragmentation >20%: 16.16% vs 10.6%; p=0.029). There was also a statistically significant decline in fertilization rate (62.5% vs. 70.07%, p=0.008), Day 3 A grade embryo formation rate (53.03% vs 70.6%; p=0.00001), Implantation rate (11.6% vs 25%; p=0.011), Clinical Pregnancy rate (15.7% vs 43.1%; p=0.025).
Limitations, reasons for caution: Multi centric studies are needed to strengthen these results.
Wider implications of the findings: We have demonstrated that poor atmospheric air during October to December in Delhi INDIA has a negative impact on embryo development which also decreases reproductive outcome even after standard air quality management. During this period either case can be avoided or more stringent air quality should be maintained.
Trial registration number: MCDH/2019/09

P-148 Does the number of oocytes affect the cumulative clinical pregnancy rate following the transfer of all frozen–thawed embryos

L. Kedar[1,2], A. Ben-Haroush[1,2], A. Wertheimer[1,2], O. Sapir[1,2], E. Shlush[1,2], Y. Shufaro[1,2], G. Oron[1,2]

[1]Belinson, Belinson Infertility and IVF Unit- Helen Schneider Hospital for Women- Rabin Medical Center- Beilinson Hospital, Petach Tikva, Israel ;
[2]Tel-Aviv University, Sackler Faculty of Medicine, Tel-Aviv, Israel

Study question: Does the number of oocytes affect the cumulative clinical pregnancy rate from the transfer of all frozen-thawed embryos.
Summary answer: The cumulative pregnancy rate from frozen-thawed embryo transfers is strongly related to the number of oocytes retrieved, regardless of the fresh transfer outcome.
What is known already: Recent studies show that the number of oocytes retrieved is correlated with the live birth rate of the fresh embryo transfer. While most studies have addressed the outcome of the fresh embryo transfer, only limited data is available on the cumulative pregnancy rate following the transfer of all fresh and frozen-thawed embryos in patients that utilized all their embryos which is the most significant outcome.
Study design, size, duration: The study included all oocyte collection cycles between January 2009 and September 2019 in a tertiary medical center that had

a fresh embryo transfer and utilized all their frozen-thawed embryos originating from the same cohort of retrieved oocytes.
Participants/materials, setting, methods: Cumulative pregnancy rate was calculated as any pregnancy from a transfer of frozen-thawed embryos originating from the same cohort of retrieved oocytes. The number of oocytes was divided into quartiles and the cumulative pregnancy rate from the frozen-thawed embryos was calculated in each group. Logistic regression analysis was utilized to adjust for potential confounders including maternal age, treatment cycle number, primary or secondary infertility, fresh embryo transfer outcome and the number of oocytes retrieved.
Main results and the role of chance: During the study period, there were 1,306 oocyte retrieval cycles that had a fresh embryo transfer and utilized all their frozen-thawed embryos originating from the same cohort of retrieved oocytes. In the first quartile (Q1) 1- 8 oocytes were retrieved (n=369), Q2 (25-50%) 9-11 oocytes (n=263), Q3 (50-75%) 12-16 oocytes (n=399) and Q4 (>75%) >17 oocytes (n=275). The cumulative pregnancy rate from frozen-thawed embryo transfers originating from the same cohort of retrieved oocytes increased with the number of oocytes retrieved Q1 76/369 (20.6%); Q2 87/263 (33.1%); Q3 137/399 (34.3%); Q4 106/275 (38.5%) p<0.001. This increase in the cumulative clinical pregnancy rate was significant with the number of oocytes retrieved among patients who conceived in the fresh transfer and (p=0.002) of those who did not (p=0.01). On a logistic regression model, maternal age and the number of oocytes were significant independent predictors for a cumulative clinical pregnancy rate from the frozen-thawed embryo transfers regardless if a pregnancy was achieved in the fresh transfer.
Limitations, reasons for caution: The limitation of the study is in the retrospective nature of the study.
Wider implications of the findings: Our findings might challenge the concept of optimal stimulation when looking at the cumulative pregnancies arising from transfer of all frozen-thawed embryos originating from the same cohort of retrieved oocytes. With agonist triggering and freeze all strategy, targeting for a higher stimulation is not only safe but also quite effective.
Trial registration number: not applicable

P-149 Deep learning model for improving embryo selection and deselection

A. Polsky[1], D.H. Silver[1], M. Feder[1], Y. Gold-Zamir[1], S. Rosentraub[1], E. Shachor[1], A. Weinberger[1], P. Mazur[2], V.D. Zukin[2], A.M. Bronstein[3]

[1]Embryonics- Ltd., Research and development, Tel Aviv, Israel ; [2]Clinic of Reproductive Medicine 'Nadiya'- Kyiv- Ukraine, Embryology, Kyiv, Ukraine ; [3]Technion – Israel Institute of Technology, Computer Science, Haifa, Israel

Study question: Can time-lapse imaging files of developing embryos be used as input for a deep learning algorithm to improve implantation probability predictions?
Summary answer: Application of time-lapse imaging files to a deep learning algorithm produced higher positive and negative predictive values than an external panel of embryologists.
What is known already: Although manual annotation and quality assessment of embryos fertilized in vitro remains the gold standard for embryo selection, efforts to improve embryo outcome prediction have become increasingly computational with research progressively turning toward artificial intelligence (AI). The majority of AI-based algorithms for embryo selection either require user-defined input parameters, or use little of the available embryo information (e.g., still images as opposed to time-lapse). Moreover, published algorithms often do not report clinically relevant performance metrics, making it difficult to transition the results from research to the clinic.
Study design, size, duration: The dataset consisted of 8,789 retrospectively collected time-lapse videos from embryos cultured to the blastocyst stage. Initial training of a machine learning (ML) model, UBar, was executed on 4,087 of the embryos, which were graded by an external panel of embryologists from various countries. Time-lapse videos were the input for the model, which was cross-validated on 272 embryos with known implantation data (KID). Prediction of implantation probability came from the cross-validation data only.
Participants/materials, setting, methods: Embryos in this study came from patients who underwent fertility treatment between 2012 and 2019 at the Clinic of Reproductive Medicine "Nadiya" in Kiev. Resulting time-lapse videos were used for developing, training, and 10-fold cross-validation of the model. Initial model training used a recurrent neural network to recover panel grades. The network was then trained to classify embryos based on their known implantation outcomes. UBar predictions were compared to embryologists' grades and implantation outcomes.
Main results and the role of chance: An encoder was trained on over 2.5 million images from unlabeled (unknown outcomes) time-lapse videos, maximizing the information available in the visual data, and "translating" it into feature vectors. A memory-based network was built to create initial predictions based on embryologists' grades. Because grades somewhat correlate with implantation success, the network used the results from graded videos to set initial feature weights. Videos from KID embryos were then used as input to train the network to predict implantation probability.
Performance of the UBar predictions exceeded that of the expert panel as calculated by the area under the receiver operating characteristic curve (AUC of the ROC): 0.82 and 0.58, respectively. For a clinically relevant performance assessment, the positive and negative predictive values (PPV and NPV) were calculated for UBar predictions and compared to those of the expert panel. Both the PPV (93%) and the NPV (58%) of UBar significantly surpassed the corresponding values of the expert panel (PPV=81% and NPV=23%), implying that application of UBar in a clinical setting could potentially improve embryo transfer outcomes. The increased NPV is particularly meaningful as an indication that UBar more accurately identifies embryos that would likely not implant.
Limitations, reasons for caution: In order to further improve results, the model will be applied to a larger amount of samples originating from multiple IVF clinics. New ML models will also be developed, and clinical parameters will be explored in future experiments to account for external factors that may influence implantation aptitude.
Wider implications of the findings: Our ML-based embryo outcome prediction model predicts implantation probability more effectively than a panel of expert embryologists. Adjunct use of UBar in IVF clinics could aid in selection of embryos with the highest implantation competence for transfer, as well as deselection of potentially non-implantable embryos.
Trial registration number: Not applicable

P-150 The impact of advanced maternal age on early and late morphokinetic parameters

O. Lebovitz[1], Y. Atzmon[1], N. Aslih[1], M. Michaeli[1], D. Paltov[1], E. Shalom-Paz[1]

[1]Hillel Yaffe Medical Center Affiliated with Rappaport Faculty of Medicine-Technion- Haifa, IVF Unit- Department of Obstetrics and Gynecology, Hadera, Israel

Study question: Are there any differences between morphokinetic parameters (MP) and embryo quality (EQ) of embryos derived from young versus advanced maternal age (AMA) women?
Summary answer: Embryos derived from younger women presented a significantly different MP in younger versus AMA women. These differences could later cause differences in EQ.
What is known already: The introduction of TLI has provided a more precise understanding of early embryo developmental stages by assigning unique quantitative identifiers to each embryo. Data regarding possible association between embryo morphokinetics and maternal age is limited and conflicting.
Study design, size, duration: Using a time-lapse incubator we retrospectively compared between MP and quality of embryos derived from young (<30 years) and AMA women (41-44 years). This study included 364 ICSI cycles (173 cycles from young and 191 from AMA) performed between January 2016 and December 2018.
Participants/materials, setting, methods: Embryo development was analyzed with TLI system (EmbryoScope, Unisense FertiliTech). The following kinetic markers were assessed: time to pronuclei fading (tPNf), and appearance of two to eight cells (t2-t8). For embryos cultured to blastocyst, time to morula (tM), start of blastulation (tSB) cavitated and expanded blastocyst (tB, tEB) were also recorded. Alpha-ESHRE and KIDScore algorithm were used to determine the transferred embryo quality. Embryos derived from young women were compared with those derived from AMA.
Main results and the role of chance: A total of 2021 oocytes were evaluated. 60.5% (n=1223) of oocytes were derived from patients under 30 years and 39.5% (n=798) from women 41 years old and above. A trend towards faster polar body disappearance observed among patients <30 years compared to patient ≥41 years old. (24.7hr vs 25.2hrs respectively, p=0.05). The mean time

points for younger patients were significantly shorter at t3, t4, t5, and t6 compared to AMA women (37.2, 39.8, 50.1 and 53.6 hrs. versus 38.4, 40.4, 51.2 and 54.7 hrs. respectively) (P<0.05). For embryos cultured to the blastocyst stage tSB, tB and tEB were significantly shorter for embryos derived from younger patients compared to embryos derived from AMA group (100.7, 108.9, 116.8 hrs. versus 104.7, 115.0, 126.6 hrs. respectively, p<0.001). There were no significant differences between the two groups at t2, t7, t8 and tM.

The proportion of toP-quality embryos (KID 4-5) was significantly higher among transferred embryos of younger women compared with AMA women (87.1% versus 76.7%; p=0.007, respectively). Similar results obtained for alpha scoring (alpha 2-3) (83.3% vs 72%; p=0.001 respectively).

Limitations, reasons for caution: Limitations of the present study are those inherent to the TLI technique which is a static system and might be operator dependent. Since the embryos cannot be rotated once entered to the embryoscope, situations like blastomeres overlap or irregular divisions might be difficult to observe by the embryologist.

Wider implications of the findings: This is the first study to compare the time-lapse analysis of younger with AMA women up to the blastocyst stages. Since increasing women's age was associated with slower embryo developmental kinetics, these parameters can be potentially utilized to improve embryo selection and cycle outcome in AMA women undergoing IVF treatment.

Trial registration number: N/A

P-151 Embryologists' perceptions following implementation of an electronic witnessing system in an in vitro fertilisation laboratory network

J. Swain[1], K. Wirka[2], B. Hayward[2], A. Catherino[2], R. Holmes[3]

[1]CCRM IVF Network, IVF Laboratory, Lone Tree, U.S.A. ;
[2]EMD Serono, Fertility & Endocrinology, Rockland, U.S.A. ;
[3]CCRM Boston, IVF Laboratory, Newton, U.S.A.

Study question: How do embryologists perceive the value of an electronic witnessing system (EWS) with regard to sample traceability, mismatch prevention, workload interruption, laboratory efficiency, system implementation and overall embryologists' confidence?

Summary answer: The EWS was well perceived by embryologists in all evaluated domains; it enhanced confidence and peace of mind at the end of a work day.

What is known already: Double witnessing has become an important additional task to prevent potential sample misidentification within the *in vitro* fertilisation (IVF) laboratory setting. Best practice dictates the use of double witnessing, but while necessary, this can be time consuming and implementation may vary between laboratories. Electronic witnessing, although not mandated in most parts of the world, has emerged as a potential way to mitigate current manual double witnessing challenges; however, embryologists' perspectives regarding the value of such technology has not been evaluated.

Study design, size, duration: This study involved administration of a web-based survey to embryology staff at eight fertility laboratories across the United States and Canada to gather attitudes and perception regarding an EWS (Gidget® [Genea BIOMEDX]). The survey remained open for 2 months. There were 73 questions related to: demographics and work experience, sample traceability, mismatch prevention, workload and interruptions, laboratory efficiencies, EWS implementation, managerial tasks and embryologist confidence.

Participants/materials, setting, methods: The questionnaire was sent to embryologists, managers and non-managers in all laboratories. Participants consented to take the anonymous survey that was composed of Likert scale statements, open-ended questions and ranking questions related to EWS. The values corresponded to categorical variables (strongly disagree, disagree, neutral, agree, strongly agree), with an option for 'no viewpoint' or 'choose not to answer' for some questions. Survey results are shown as proportion agreeing with statements and 95% confidence intervals (CI).

Main results and the role of chance: The survey response rate was 96.2% (50/52). Mean (±standard deviation) experience in a clinical IVF setting was 12.94 (±8.67) years for all embryologists (n=45) and 18.67 (±6.79) years for the subset of managers (n=15); experience with the EWS was 10.9 (±6.30) months and 12.4 [±5.47] months, respectively. Overall (% [95% CI]), 78.3 (63.6, 89.1)% of respondents agreed that EWS improved sample traceability and 80.4 (66.1, 90.6)% agreed that EWS reduced labelling errors. Just 60.9 (45.4, 74.9)% agreed that EWS reduced the risk of sample mismatch errors by minimising disruptions. Visual completion of the dashboard provided peace of mind when leaving work to 82.6 (68.6, 92.2)% of respondents, and 84.8 (71.1, 93.7)% felt more confident knowing that procedures were completed according to the EWS. Most respondents (73.9 [58.9, 85.7]%) agreed that EWS allowed double witnessing to be conducted quickly and accurately, and that labelling standardization increased efficiency (84.8 [71.1, 93.7]%). The majority of respondents considered EWS training easy (78.3 [63.6, 89.1]%) and would recommend the EWS to another embryologist (88.9 [75.9, 96.3]%), while 26.1 (14.3, 41.1)% had concerns with transitioning from manual to EWS. Managers agreed (93.3 [68.1, 99.8]%) that sample chain of custody was easily followed using EWS audit reports.

Limitations, reasons for caution: The survey was sent to one network of embryology laboratories as it relates to a single EWS. Embryologists' perceptions may be influenced by length of experience with a particular system and by their general embryology experience and knowledge.

Wider implications of the findings: This study indicates that the implementation of an EWS was well perceived by most embryologists. Sample identification, traceability, mismatch prevention, laboratory efficiency and embryologist confidence was perceived to be improved. For the first time, these data captured embryologists' opinion on the value of an EWS.

Trial registration number: NA

P-152 Multinucleation has no impact on an embryos ability to develop, implant or sustain a viable pregnancy to live birth

V. Songkhunawej[1], L. Burmeister[2], K. Sorby[1]

[1]Number 1 Fertility, Embryology, East Melbourne, Australia ;
[2]Number 1 Fertility, Clinician, East Melbourne, Australia

Study question: The aim of our study was to investigate the impact of multinucleation on ART outcomes including embryo development, utilisation, pregnancy loss and live birth.

Summary answer: Presence of multinucleation appears to have no negative impact on embryo development nor the embryo's ability to implant and sustain a viable pregnancy.

What is known already: Detected as early as 1987 by Tesarik et al, multinucleation in embryos is thought to arise from abnormalities in DNA synthesis during cleavage. Previous literature demonstrated that multinucleation in embryos was linked to increased aneuploidy rate (Kligman et al, 1996) and lower implantation and live birth rate (Hardarson et al., 2001). However, development of time-lapse technology has dramatically improved embryo assessment from just a static timepoint to continuous monitoring, enabling more robust detection of multinucleation. Recent studies suggest that ploidy (Balakier et al., 2016) in addition to perinatal outcomes (Seikkula et al., 2018) are not impacted by multinucleation.

Study design, size, duration: In this retrospective study, data was collected from cycles dating from 1st February 2018 to 31st December 2019. In this period, there were a total of 8409 embryos assessed. To date, pregnancy and live birth data were available for 363 embryos.

Participants/materials, setting, methods: Embryos were cultured in the Embryoscope+ to day 5 or 6 and assessed for routine morphokinetic parameters. Multinucleation assessment included: presence or absence at the 2-cell and 4-cell stages, number of blastomeres affected, number of nuclei seen. Utilisation, clinical pregnancy and live birth rates were assessed. Transfers were single day 5 embryos. To simulate observation of multinucleation in laboratories not using time-lapse, multinucleation was assessed in eleven planes at the static timepoint of 42 hours.

Main results and the role of chance: Multinucleation was seen in 31.77% of embryos under time-lapse. However, when assessing at 42 hours, only 8.1% of embryos had visible multinucleation. The occurrence of multinucleation was more likely at the two cell stage in comparison to the four cell stage (*20.60% vs 6.20%; p = 0.001*) with 4.98% of embryos having multinucleation persisting through both the two and four cell stages. Prevalance of multinucleation was not impacted by age, presence of smooth endoplasmic reticulum discs, uneven pronuclei, uneven two cell blastomeres or ploidy. Time to cavitation (both absolute and relative to syngamy) which was used as a marker of embryo development, did not appear to be impacted by the presence of multinucleation (*78.10h vs 78.34h; p = 0.0585*) as well as embryo utilisation (*51.03% v 47.05%; p = 0.8731*). The clinical pregnancy rate was no different when comparing embryos with or without multinucleation (*30.12% v 26.77% respectively; p = 0.4848*). Reassuringly, the same was seen in the pregnancy loss rate where non-multinucleated embryos had a rate of 23.61% versus those with

multinucleation at 16.00% (*p = 0.5761*). Furthermore, birth weight and gender ratios were not significantly different between groups.
Limitations, reasons for caution: Despite having a large sample size for our main analysis, after filtering down to embryos that were transferred and had live birth data available, a larger sample size would be more desirable for birth outcomes. Interobserver variability is also likely, due to multiple embryologists making embryo assessments.
Wider implications of the findings: Multinucleation does not appear to have an impact on the capacity of the embryo to develop to blastocyst, implant or sustain a viable pregnancy to live birth. Multinucleation appears to be an insignificant factor in the developing embryo and should not be used as a criteria for stratification of embryos.
Trial registration number: Not Applicable

P-153 Improving the performance of deep convolutional neural networks (CNN) in embryology using synthetic machine-generated images

M. Kanakasabapathy[1], C. Bormann[2], P. Thirumalaraju[1], R. Banerjee[1], H. Shafiee[3]

[1]Brigham and Women's Hospital, Division of Engineering in Medicine, Boston, U.S.A. ; [2]Massachusetts General Hospital, Obstetrics and Gynecology, Boston, U.S.A. ; [3]Brigham and Women's Hospital, Department of Medicine- Harvard Medical School, Boston, U.S.A.

Study question: Can we employ synthetic machine-generated images in empowering AI-based morphological assessment of cells with applications in embryology?
Summary answer: The addition of synthetic oocyte images significantly improved the performance of a CNN in oocyte assessment compared to a CNN trained on clinical oocyte images.
What is known already: CNNs have shown an enormous potential in embryology. These neural networks, in general, require large amounts of expertly annotated data to perform sufficiently well. However, the availability of datasets is limited to very few fertility centers worldwide. Generative adversarial networks (GANs), which excel in generating life-like images, may help in mitigating such data requirements. It has been shown previously that oocytes can be differentiated based on their potential for fertilization by CNNs through morphological analysis (Thirumalaraju et al., 2019). Here, we evaluate whether such a CNN when trained with additional data generated by a GAN can improve its performance.
Study design, size, duration: Using a retrospective dataset of clinical oocyte images with known fertilization outcomes (KFO) and synthetic oocyte images generated by a pretrained GAN, a CNN, henceforth called synthetic CNN (s-CNN), was trained to classify between oocytes that eventually fertilized normally, with 2 pronuclei (2PN), and abnormally (non-2PN). The network performance was compared to the performance of a CNN, henceforth called the original CNN (o-CNN), trained using clinical oocyte images only.
Participants/materials, setting, methods: A GAN was trained to generate life-like oocyte images from scratch. The s-CNN was trained using a dataset of 1411 clinical oocytes images with KFO and 1340 synthetic oocyte images generated by the GAN. The o-CNNs were trained as reported previously using only the 1411 clinical oocyte images with KFO. The trained networks were tested with a common set of 712 clinical oocyte images with KFO.
Main results and the role of chance: The o-CNN performed with an accuracy of 67.0% (95% CI: 63.4% to 70.4%) and an AUC of 0.6133 in classifying oocytes based on their eventual fertilization outcome, as reported previously (n=712). In contrast, the s-CNN performed with an improved accuracy of 82.58% (CI: 79.59% to 85.30%) and an AUC of 0.8123 (n=712) in differentiating the oocyte images based on their eventual fertilization outcomes.
Limitations, reasons for caution: Images were obtained using a single imaging platform (EmbryoScope) at a single timepoint. Fertilization assessments (2PN evaluations) we performed by individual embryologists from a single center.
Wider implications of the findings: The training with GAN-generated data helped s-CNN to outperform the conventionally trained o-CNN when evaluating real oocytes with KFO. Thus, GANs may hold the potential to improve the currently utilized CNNs in embryology.
Trial registration number: not applicable

P-154 Piezo-ICSI can produce significantly higher fertilization and blastocyst rates without increasing the risk of birth defects as compared to the IVF

K. Hiraoka[1], M. Ibayashi[1], A. Kuga[1], N. Uchida[1], T. Sujino[1], K. Kawai[1]

[1]Kameda IVF Clinic Makuhari, ART Laboratory, Chiba, Japan

Study question: Can Piezo-ICSI increase fertilization and blastocyst rates without increasing the risk of birth defects as compared to the IVF?
Summary answer: Piezo-ICSI can significantly increase fertilization and blastocyst rates without increasing the risk of birth defects as compared to the IVF
What is known already: Recently, the clinical usefulness of Piezo-ICSI has been reported by some investigators. All papers reported significantly higher survival or fertilization rates and embryo development in Piezo-ICSI as compared to conventional-ICSI. On the other hand, some papers reported that the comparison of conventional-ICSI and IVF children taking part in an identical follow-up study did not show any increased risk of birth defects and neonatal complications in the conventional-ICSI group. To the best of our knowledge, there is no paper comparing the ICSI results, embryo development and delivery outcome between Piezo-ICSI and IVF.
Study design, size, duration:

1. We retrospectively investigated 6,671 mature oocytes between May 2016 and December 2019. Of these, 3,156 mature oocytes were inseminated by IVF and 3,516 mature oocytes were inseminated by Piezo-ICSI and the ICSI results and embryo development were compared.
2. We retrospectively investigated 204 infants born after our IVF program since May 2016. Of these, 82 infants were derived from IVF and 122 infants were derived from Piezo-ICSI and the delivery outcomes were compared.

Participants/materials, setting, methods:

1. The fertilization, blastocyst and good quality blastocyst rates of IVF and Piezo-ICSI were compared.
2. The number of embryos transferred, monozygotic twin rates, dizygotic twin rates, gestational week, birth weight, the ratio of boy and girl and birth defects rates were compared.

The good quality blastocyst was defined as better than grade BB by Gardner's criteria. The data were analyzed by Unpaired T Student, chi-square test or Fisher's exact test.
Main results and the role of chance:

1. Among the IVF and Piezo-ICSI, there were no significant differences when comparing the average age of women at the insemination (34.3 ± 4.1 vs. 34.7 ± 4.1). The fertilization rates of IVF and Piezo-ICSI were 73.0% and 83.8%. A significantly higher fertilization rate was observed in Piezo-ICSI ($P < 0.01$). The blastocyst rates of IVF and Piezo-ICSI were 41.3% and 46.2%. A significantly higher blastocyst rate was observed in Piezo-ICSI ($P < 0.01$). The good quality blastocyst rates of IVF and Piezo-ICSI were 19.1% and 22.4%. A significantly higher blastocyst rate was observed in Piezo-ICSI ($P < 0.01$).
2. Among the IVF and Piezo-ICSI, there were no significant differences when comparing the average age of women at the embryo transfer (34.6 ± 4.0 vs. 35.1 ± 4.1), average number of embryos transferred (1.0 ± 0.3 vs. 1.1 ± 0.3), monozygotic twin rates (1.3% vs. 1.7%), dizygotic twin rates (1.3% vs. 0.8%), gestational week (38.8 ± 2.8 vs. 38.7 ± 2.7), birth weight (3036 ± 1751g vs. 3005 ± 1662g), the ratio of boy and girl (51:49 vs. 49:51) and birth defect rates (5.0% vs. 3.3%).

Data are expressed as mean ± SD.
Limitations, reasons for caution: The number of matured oocytes of IVF was calculated as the total number of 0PN (with no pronucleus and with1 or 2 polar bodies), 1PN (mono-nucleus), 2PN (two-pronucleus), ≥3PN (more than three-pronucleus) at the time of fertilization assessment. So, the 0PN oocytes might be immature at the time of insemination.

Wider implications of the findings: Piezo-ICSI can produce significantly higher fertilization and blastocyst rates without increasing the risk of birth defects as compared to the IVF. Therefore, Piezo-ICSI could be the main insemination method for human assisted reproductive technology to improve the effective utilization rate of precious oocytes.
Trial registration number: not applicable

P-155 Vitamin D (VitD) in follicular fluid (FF) correlates with the euploid status of the embryo in a VitD deficient Middle Eastern population.

A. Arnanz[1], N. De Munck[1], I. El Khatib[1], A. Bayram[1], A. El-Damen[1], A. Abdala[1], L. Melado[2], B. Lawrenz[2], A. Pacheco[3], H. Fatemi[2]

[1]IVIRMA Middle East Fertility Clinic LLC, IVF lab, abu dhabi, United Arab Emirates ; [2]IVIRMA Middle East Fertility Clinic LLC, Gynaecology, abu dhabi, United Arab Emirates ; [3]IVIRMA Madrid, Andrology, Madrid, Spain

Study question: Is there a correlation between VitD levels in individual FF and embryo ploidy status among Middle Eastern patients undergoing in vitro fertilization (IVF) treatments?
Summary answer: In a Vitamin D deficient population, euploid blastocysts showed significantly higher levels of 25-hydroxyvitamin D[25(OH)D] and bioavailable 25(OH)D in FF compared to the aneuploid ones.
What is known already: The widespread distribution of the VitD receptor in reproductive tissues suggests an important role for VitD in human reproduction. Despite the abundance of sunlight, due to socio cultural/religious habits, the prevalence of VitD deficiency/insufficiency in Middle Eastern women is known to be among the highest in the world.

The assessment of patient´s VitD is based on the 25(OH)D metabolite measurement. However, most of the circulating 25(OH)D is bound to either vitamin D-binding protein (VDBP) (88%) or albumin (12%) and less than 1% circulates free. The albumin-bound fraction of 25(OH)D plus free fraction equals the bioavailable fraction of 25(OH)D.
Study design, size, duration: A prospective observational study was performed between 2017 and 2019, including Middle Eastern couples planned for preimplantation genetic testing for aneuploidies (PGT-A). Trophectoderm biopsy was performed on 115 blastocysts from 37 couples and subjected to Next Generation Sequencing. Serum (S) samples were withdrawn on the day of the final oocyte maturation and 115 FF samples from follicles ≥14mm were individually collected during the oocyte retrieval for 25(OH)D, bioavailable 25(OH)D and free 25(OH)D measurement.
Participants/materials, setting, methods: Patients were classified into two groups according to the serum 25(OH)D levels; VitD deficient <20 ng/ml and insufficient/replete ≥20 ng/ml.

Quantitative measurement of total 25(OH)D was performed using the Roche total 25(OH)D assay with competition principle in an electrochemiluminescence (ECLIA) binding assay. VDBP was measured using an ELISA kit (R&D system). Free and bioavailable 25(OH)D were determined using the method reported by Bikle et al (1986) applying a modified Vermeulen formula.
Main results and the role of chance: The average maternal age in both groups was 31.1±6.0 vs 32.1±5.0; p=0.369, body mass index 26.1±2.9 vs 25.6±3.8; p=0.499, Antimullerian Hormone 3.8±2.2 vs 3.5±1.4; p=0.400, mature oocytes 14.2±6.9 vs 13.0±4.9; p=0.561, respectively in <20 ng/ml and ≥20 ng/ml group. Serum values were 13.2±4.0 vs 32.3±9.2; p<.0001, respectively. FF samples from a total of 40 and 75 blastocysts were analyzed of which 52.5% (21/40) and 61.3% (46/75) were euploid, respectively.

In <20 ng/ml group, the logistic regression showed that the average values of 25(OH)D and bioavailable 25(OH)D were significantly higher in follicles resulting in euploid vs aneuploid blastocysts (18.3 vs 13.9; p=0.029) vs (18.1 vs 13.7; p=0.029). However, in ≥20 ng/ml group, no significant differences were found (38.1 vs 40.6; p=0.417) and (37.7 vs 40.1; p=0.441), respectively.

A Pearson test (ρ) indicated a strong correlation between S and FF 25(OH)D, bioavailable 25(OH)D and free 25(OH)D values (r= 0.805 vs r= 0.805 vs r=0.834; p<.001 respectively).

In a multivariate model, confounding for age, BMI and AMH, sufficient/replete have a significantly higher probability to have a euploid embryo compared to deficient patients (Estimate:1.64, p=0.031). Moreover, in the deficient group, age was significantly higher for aneuploid blastocysts (33.3±5.5 vs 29.2±5.8; p=0.03).
Limitations, reasons for caution: This study is based on a good prognosis Middle Eastern population, who are mainly VitD deficient or insufficient (<30 ng/ml), therefore these results can not be extrapolated to other patient groups.
Wider implications of the findings: In a deficient VitD population, intrafollicular concentration of VitD is a marker of embryo competence. The mechanism of the impact of VitD on chromosomal status of the blastocysts needs to be further investigated. Preliminary data in the literature suggest that telomere length may be involved.
Trial registration number: NCT03073720

P-156 Piezo-ICSI can generate significantly higher survival, fertilization and blastocyst rates without increasing the risk of malformation as compared to the Conventional-ICSI

A. Kuga[1], K. Hiraoka[1], M. Ibayashi[1], N. Uchida[1], T. Suhara[2], T. Kaji[2], Y. Kamada[2], T. Sujino[1], K. Ohuchi[3], M. Hayashi[3], K. Kawai[4]

[1]Kameda IVF Clinic Makuhari, laboratory, Chiba, Japan ; [2]Kameda Medical Center, laboratory, Chiba, Japan ; [3]Kameda Medical Center, ART center, Chiba, Japan ; [4]Kameda IVF Clinic Makuhari, ART center, Chiba, Japan

Study question: Can Piezo-ICSI generate higher survival, fertilization and blastocyst rates without increasing the risk of malformation as compared with the Conventional-ICSI?
Summary answer: Piezo-ICSI can generate significantly higher survival, fertilization and blastocyst rates without increasing the risk of malformation as compared with the Conventional-ICSI.
What is known already: ICSI has been widely performed by using micropipette with spiking tip and usually, the cytoplasm is aspirated to break the membrane (Conventional-ICSI). Recently, the clinical usefulness of Piezo-ICSI has been reported by several investigators. In Piezo-ICSI, micropipette with flat tip is used and the membrane breakage is performed by applying piezo without aspirating the cytoplasm into the micropipette. When comparing the survival and fertilization rates between Conventional-ICSI and Piezo-ICSI, favorable results of Piezo-ICSI has been reported. However, little information is available comparing the ICSI results and delivery outcome between Conventional-ICSI and Piezo-ICSI.
Study design, size, duration:

1. We retrospectively investigated 5,748 mature oocytes between January 2012 and December 2019. Of these, 1,538 mature oocytes were inseminated by Conventional-ICSI and 4,210 mature oocytes were inseminated by Piezo-ICSI and the ICSI results and embryo development were compared.
2. We retrospectively investigated 178 infants born after our IVF program since January 2012. Of these, 89 infants were derived from Conventional-IVF and 89 infants were derived from Piezo-ICSI and the delivery outcomes were compared.

Participants/materials, setting, methods:

1. The survival, fertilization and blastocyst rates of Conventional-ICSI and Piezo-ICSI were compared.
2. The number of embryos transferred, monozygotic twin rates, dizygotic twin rates, gestational week, birth weight, the ratio of boy and girl and malformation rates were compared.

The data were analyzed by Unpaired T Student, chi-square test or Fisher's exact test.
Main results and the role of chance:

1. The survival rates of Conventional-ICSI and Piezo-ICSI were 92.9% and 95.2%. A significantly higher survival rate was observed in Piezo-ICSI ($P < 0.01$). The fertilization rates of Conventional-ICSI and Piezo-ICSI were 69.9% and 81.1%. A significantly higher fertilization rate was observed in Piezo-ICSI ($P < 0.01$). The blastocyst rates of Conventional-ICSI and Piezo-ICSI were 47.1% and 54.4%. A significantly higher blastocyst rate was observed in Piezo-ICSI ($P < 0.01$).

2. Among the Conventional-ICSI and Piezo-ICSI, there were no significant differences when comparing the average age of women at the embryo transfer (38.4 ± 4.3 vs. 38.7 ± 4.0), number of embryos transferred (1.2 ± 0.4 vs. 1.2 ± 0.4), monozygotic twin rates (4.7% vs. 0%), dizygotic twin rates (0% vs. 4.7%), gestational week (38.2 ± 1.7 vs. 38.3 ± 2.1), birth weight (2913 ± 462g vs. 2947 ± 489g), the ratio of boy and girl (38:62 vs. 53:47) and malformation rates (6.7% vs. 6.7%).

Data are expressed as mean ± SD.
Limitations, reasons for caution: In our IVF program, day 2 or 3 after oocyte retrieval, 1-3 good quality cleaved embryos were cryopreserved or transferred, so the blastocyst culture was performed excluding these embryos.
Wider implications of the findings: Piezo-ICSI can generate significantly higher survival, fertilization and blastocyst rates without increasing the risk of malformation as compared to the Conventional-ICSI. Therefore, Piezo-ICSI could be an alternative ICSI method to the Conventional-ICSI without losing valuable oocytes for human assisted reproductive technology.
Trial registration number: not applicable

P-157 Optimum timing of trophectoderm biopsy with cryopreservation for preimplantation genetic testing for aneuploidies (PGT-A) cycle

E.J. Yu[1], E.A. Park[1], S.A. Choe[1], M.J. Kim[1], Y.S. Kim[1], J.Y. Lee[1], E.B. Joe[1], H.S. Kim[1], J.H. Lee[1], H.O. Kim[1], Y.S. Kim[1], M.K. Koong[1], I.S. Kang[1], T.K. Yoon[1]

[1]CHA Fertility Center- Seoul Station, Department of Obstetrics and Gynecology, Seoul, Korea- South

Study question: Is the timing of trophectoderm(TE) biopsy before or after vitrification-warming associated with successful clinical outcomes after the embryo transfer(ET) of blastocyst subjected to preimplantation genetic testing for aneuploidies (PGT-A)?
Summary answer: TE biopsy(PGT-A) with cryopreservation may be the optimal timing for better clinical outcomes compared to the fresh TE biopsy without cryopreservation.
What is known already: Embryo biopsy and fresh embryo transfer are traditionally performed in the PGT-A cycle. However, before ET, the time allowed for genetic analysis of the specimens is restricted, particularly after blastocyst biopsy. Cryopreservation of blastocysts after biopsy instead of fresh transfer permits more sufficient time for performance of molecular diagnosis. The effect of cryopreservation and warming procedures on clinical outcomes in PGT-A cycle has not been effectively studied.
Study design, size, duration: Retrospective analysis of patients with PGT-A cycles was carried out from January 2016 to November 2019. 3684 blastocysts from cycles were subjected to TE biopsy for performing array comparative genomic hybridization test. Embryos were cultured to expanded blastocyst and performed TE biopsy on day 5 or day 6, depending on the timing of embryo expansion. Cycles with complete PGT-A diagnosis were 655, among them 349 of which had ET.
Participants/materials, setting, methods: The performance of different groups of PGT-A patients was evaluated. The groups were divided into three; first group (n=118 transfer/677 cases) contained fresh blastocysts that biopsied for PGT-A without cryopreservation followed by embryo transfer. In the second group (n=348 transfer/355 cases), the blastocysts that were biopsied prior to vitrification; and subsequently warmed euploid blastocysts for ET. The last group (n=189 transfer/248 cases), the vitrified blastocysts were warmed and then biopsied before proceeding with ET.
Main results and the role of chance: The total pregnancy and implantation rates of fresh blastocyst biopsied group was 42.4% (50/118), 35.7%(56/157); the cryopreserved-warmed-biopsied blastocyst group showed 56.6% (197/348), 51.2% (227/443); and finally biopsied and cryopreserved-warmed group showed 54.0% (102/189), 48.6% (123/253); respectively. Second group and third group are significant higher pregnancy rates than first group. Also, The second(biopsied and cryopreserved-warmed) group and third (cryopreserved-warmed–biopsied) group are significant higher implantation rates than first group. Also, The second group(36/197, 18.3%) showed higher numerical miscarriage rate than the first group(6/50, 12.0%) and third group(16/102, 15.7%).
Limitations, reasons for caution: In the case of biopsy after warming, it is difficult to determine the number of warming embryos and re-cryopreservation of surplus euploid embryo could increase embryo damage and miscarriage rate.
Wider implications of the findings: Using recently available data, when faced with the option of fresh embryos, before or after trophectoderm biopsy for PGT-A, our result supported performing TE biopsy of blastocyst for PGT-A before or after vitrification and warming may be the optimal timing for better clinical outcomes.
Trial registration number: Not applicable

P-158 A machine-learning (ml) based decision support framework with a case study in frozen-embryo-transfer (fet) in-vitro-fertilization (ivf) for patients with polycystic ovarian syndrome (pcos)

T.J Ramnath Babu[1], V.S. Roy[2], Y. Jambunath[1], D. Modi[3], S.B. Bhuvarahamurthi[1], N. T s[4]

[1]SpOvum Technologies Pvt. Ltd., Product Development, Bangalore, India ;
[2]Manipal Fertility - JP Nagar, Scientific Director & Chief Embryologist, Bangalore, India ;
[3]National Institute For Research In Reproductive Health ICMR-NIRRH, Molecular and Cellular Biology Lab, Bangalore, India ;
[4]Manipal Fertility - JP Nagar, Clinical Director, Bangalore, India

Study question: Can a data-driven framework not just predict, but also aid embryologists/ clinicians in decision-making during a FET-IVF treatment for patients with PCOS?
Summary answer: We show that one can augment the capability of prediction-tools and controllability of parameters using a ML-based framework towards improving 'clinical pregnancy rate' (CPR).
What is known already: Existing literature primarily focus on prediction, and seldom aid in the IVF treatment process. Recent decision-support systems in the IVF domain consider non-clinical parameters like demographic details. However, the efficacy of the decisions taken during the treatment are not considered. It is also noted that earlier case studies cover diverse population including several male and female causes of infertility, to predict IVF outcomes. One downside of including miscellaneous collection of infertility factors is that the model becomes highly uncertain. Specifically, no study has covered PCOS condition, the most common cause of infertility.
Study design, size, duration: We developed a supervised machine learning model covering only PCOS in FET-IVF cycle. The purpose of the study is to classify the CPR as either Yes/No. Anonymized clinical data along with CPR outcomes of 330 PCOS patients who underwent FET-IVF treatment at an IVF center between 2015 and 2019 were retrospectively analyzed. Patients with donor samples (donor oocyte/ sperm/ embryo), severe male infertility factors were excluded from this dataset, resulting in 187 (~56%) patient records
Participants/materials, setting, methods: We avoided sampling to reduce uncertainty. Feature engineering was performed to label the details including embryo details (quantity and grade of embryos before freezing, post thawing and before embryo transfer) as good or moderate or poor. Then we used naïve Bayes algorithm to classify CPR as Yes or No. Here, CPR as 'Yes' is defined as the ultrasound visualization of a pregnancy sac with fetal heart activity from six weeks after frozen embryo transfer.
Main results and the role of chance: The f-value statistical test results suggested that all the three parameters (good (~1) / moderate (~1.5)/ poor (~7)) related to the embryo grades are more correlated with CPR compared to other parameters. Our embryo-grades representation is intuitive and generic and can be performed across all clinics. For decision making, the embryologist's inputs are included as parameters (features) to the model.

A naïve Bayes classifier model was developed using 95% training dataset with seven features, and prediction performance were evaluated using macro f1-score and accuracy on the remaining 5%. Macro f1-score was calculated as the harmonic mean of precision and recall of each class label (Yes, No), hence reducing skewness in prediction.

Model predictions using test data resulted in 90% accuracy and 90% f1 score at best.

To demonstrate the role of the ML framework, we chose 'Day of freezing', 'Culture type' and 'Number of embryos transferred' as the controllable parameters for decision-making. The model calculates all scientifically possible

combinations of these parameters and ranks the suggestions with confidence scores. An embryologist can decide the pathway of the treatment using this rank list.
Limitations, reasons for caution: A limitation in this study was the small size of dataset collected from a clinic. This limitation could be overcome by using more data from multiple clinics, thus improving the model's performance.
Wider implications of the findings: The ML framework shall be able to support and guide embryologists on the different paths for them to plan and adhere-to in order to achieve better success rates. The framework also sensitizes the criticality of certain data-points which will further impact the perspective of an IVF treatment.
Trial registration number: Not applicable

P-159 Oocyte maturation changes mitochondrial gene expression patterns and the localization of polarized mitochondria but does not affect mitochondrial potential

F. Zambelli[1], M. Barragan[1], R. Vassena[1]

[1]Clinica Eugin, Research and Development, Barcelona, Spain

Study question: Are there changes in the oocyte mitochondrial transcriptome and membrane potential during meiotic maturation?
Summary answer: We identified a decreased mitochondrial gene expression and a more compact distribution of polarized mitochondria in MII compared to GV; mitochondrial membrane potential was unaffected
What is known already: Mitochondria localization and activity in oocytes from several species has been associated with the acquisition of developmental potential. Several studies have attempted to associate mitochondrial measurements such as DNA copy number and gene expression in human oocytes with reproductive outcomes after IVF. These tests rely on the assumption that a reduction in the abundance of certain transcripts directly translates to a modification in mitochondrial activity. However, this correspondence has not yet been proven. Moreover, a clear causation between an altered mitochondrial activity and the failure to reach MII in human oocytes hasn't been demonstrated yet.
Study design, size, duration: We analyzed the mitochondrial membrane potential and the localization of active mitochondria in human oocytes at GV, GV after 30 hours in culture (FTM-GV), and MII from GV after 30 hours in culture (IVM-MII). Further, we performed a transcriptomic-wide analysis via microarray of GV and in vivo matured MIIs (IVO-MII) to correlate functional and transcriptional measurements.
Participants/materials, setting, methods: 24 oocyte donors (ages 20 to 35) provided 63 oocytes (24 GV, 15 FTM-GV, 15 IVM-MII, 9 IVO-MII) for the study. Oocytes were stained with JC-1 to identify both polarized and non-polarized mitochondria and imaged by confocal microscopy. Intensities of the staining and spatial measurement of the polarized mitochondria layer were performed using ImageJ. Transcriptome-wide analysis via microarray (HTA 2.0 Affymetrix) was performed using 4 GVs and 9 in vivo matured MII
Main results and the role of chance: GV and IVO-MII oocytes presented 543 transcripts differentially expressed (516 more abundant in GV, 27 more abundant in MII). Oxidative phosphorylation and mitochondrial function were the most affected pathways by Gene Ontology analysis, with a significant decrease of mitochondrial transcripts like ATP5, PDHA, and POLG in MIIs. JC-1 staining indicated in all groups a rather thin layer of polarized mitochondria at the periphery of the oocyte (within 20 µm from the oolemma), while the inner part of the oocyte (>20 µm from the oolemma) only contained non-polarized mitochondria. We found no differences in the membrane potential of the peripheral mitochondria across groups, (39% increase in polarized mitochondria in FTM-GV, 15% increase in polarized mitochondria in IVM-MII compared to GV; $p>0.05$). We did observed differences in the distribution of mitochondria between GV, FTM-GV and IVM-MII. Both GV and FTM-GV had a wider distribution of the polarized mitochondria (9.7 ± 1.7 µm and 7.9 ± 1.5 µm, respectively), while in IVM-MII we observed a thinner layer (6.2 ± 1.7 µm), placed in the outermost part of the ooplasm ($p<0.001$).
Limitations, reasons for caution: The origin of MII (both in vitro and in vivo matured) may affect some of the comparative observations; GVs were collected after controlled ovarian stimulation and their transcriptome may be different from GVs from unstimulated ovaries.
Wider implications of the findings: Our results might inform the development of better culture media, where the presence of mitochondrial modulators might improve oocyte quality. Further, the differences between transcription and mitochondrial activity may be used to develop reliable predictive test for mitochondrial genetic screening
Trial registration number: not applicable

P-160 Remote embryo assessment: shifting the paradigm from traditional embryo grading

A. Picou[1], K.A. Wirka[2], A.B. Catherino[2], B. Hayward[2], M.D. VerMilyea[1,3,4]

[1]Ovation Fertility, Laboratory and Operations, Austin, U.S.A. ;
[2]EMD Serono, Fertility and Endocrinology, Rockland, U.S.A. ;
[3]Ovation Fertility, IVF Lab, Austin, U.S.A. ;
[4]Texas Fertility Center, IVF Laboratory, Austin, U.S.A.

Study question: How confident are embryologists with remote embryo assessment (REA) using time-lapse (TL) technology as an alternative to traditional embryo assessment (TEA) when performing morphology grading?
Summary answer: REA enables morphology grading with high confidence at fertilization assessment (Day 1) and Days 3, 5, 6 and 7 of embryo development.
What is known already: Despite other advances in assisted reproductive technology, morphology is the stalwart used by embryologists to grade embryos for transfer. To be able to assess and select embryos with minimal culture disturbance is highly desirable. The impact of REA enabled by TL technology in the *in vitro* fertilization (IVF) laboratory remains unknown, including its potential impact on embryo grading and selection compared with TEA (routinely performed with a light microscope). Most importantly, the level of confidence in embryo assessments performed remotely by embryologists using this novel technology has not been previously evaluated. Here we describe preliminary results of a feasibility study.
Study design, size, duration: Non-interventional, longitudinal study in a single IVF centre over 5 months. Seven embryologists performed Day 1–7 morphology gradings using TEA and REA (Geri Connect and Assess® [Genea BIOMEDX]). Embryologists rated their level of confidence (high/low) for each embryo grading and stated reasons for low confidence, when applicable. TEA was performed using standard procedures and REA performed after a ≥1 week washout period. This study was determined exempt from review by Sterling IRB, Atlanta, GA, USA.
Participants/materials, setting, methods: Data included 5458 morphology gradings from 72 embryo cohorts evaluated on Days 1,3,5,6 and 7. TL imaging was initiated on Day 0. TEA/REA were timed and performed by the same embryologist; elapsed recording time was used to match timing of TEA (performed in real time) to the retrospective REA (performed elsewhere at their convenience). REA Block I had no time restrictions; Block II simulated TEA's time sensitivity. Confidence in gradings and video usage were reported.
Main results and the role of chance: Mean durations (minutes ± standard deviation) of cohort assessments were TEA 2.14 (±1.30) and REA 2.02 (±1.18) with an average of 10.1 embryos per cohort. Embryologists reported high confidence in 94.4% (636/674) of Day 1 TEA-graded zygotes. REA matched high confidence in 88.1% (560/636). High confidence was reported for 98.5% (585/594) of Day 3 and 96.0% (1308/1363) of Day 5–7 TEA gradings; REA matched in 94.4% (552/585) and 95.0% (1242/1308), respectively. Among embryos graded with low confidence using TEA, 44.7% (17/38) of Day 1, 66.7% (6/9) of Day 3 and 72.7% (40/55) of Day 5–7 were graded with high confidence using REA. During REA Block I and II, video review was used for 5.1% (53/1033) and 0.5% (8/1666) of embryos, respectively, with 91.8% (56/61) of embryologists having improvement in confidence. The main low confidence reasons reported for TEA were "not able to roll embryo around" (56.0% [61/109]; mostly for Day 1, 59.0% [36/61]) and "need for a second opinion" (25.7% [28/109], all Day 5–7). Within the REA group, "poor focus or focal range" (55.9% [123/220], caused by device malfunction in 25.2% [31/123]) and "not able to roll embryo around" (23.2% [51/220]; mostly for Day 1, 54.9% [28/51]).
Limitations, reasons for caution: This study was conducted in a single clinic using a specific TL device and REA system; clinics using different devices and systems may not observe the same results.
Wider implications of the findings: This is the first study to evaluate embryologist confidence in embryo grading using a remote system. REA embryo gradings can be performed with high confidence compared with traditional assessment. Improved embryologist confidence for embryos previously graded

with low confidence may be associated with image replay and review enabled by REA.
Trial registration number: N/A

P-161 The effect of high humidity by using single step culture media on continuous embryo monitoring incubator.

C. Albert Rodriguez[1], M.A. Valera[1], S. Pérez-Albalá[1], L. Alegre Ferri[1], B. Aparicio-Ruiz[1], J. Remohí[2], M. Meseguer Escrivá[1]

[1]IVIRMA, Lab FIV, Valencia, Spain ;
[2]IVIRMA, Ob/Gym, Valencia, Spain

Study question: Is there any effect of high humidity culture conditions by using single step Media and Continiuous Embryo Monitoring (CEM) on reproductive outcome?
Summary answer: By using single step media and CEM incubator Geri, culturing embryos under high humidity does increase reproductive outcome
What is known already: The first goal of an IVF laboratory is to achieve stable conditions to allow the best embryo development. An incubator has control different variables, like temperature, gas. Usually we don´t need to use the humid conditions, as oil overlay has supported successful use of a dry incubator for culture human embryos, preventing changes in the pH, temperature. The evaporation of culture media may affect the osmolality. Therefore, the use of humid conditions avoid osmolality changes.
Study design, size, duration: A total of 5755 embryos from ovum donation programme and own oocytes and that were culture on a CEM system (Geri genea biomedix, Australia) were included in a retrospective and multicentric study from three consecutive years.
Participants/materials, setting, methods: This incubator has 6 separated incubators. Three of them works in a dry atmosphere (DC) and the other 3 in humid conditions (HC). In DC, 2630 embryos were cultured and a total of 3125 embryos were cultured under HC. Retrospectively, blastocyst, good morphology blastocyst rate, pregnancy, and euplody rate.
Main results and the role of chance: We found differences in embryo development. We had similar blastocyst rate when the embryos were culture under high humidity; 52.8% vs DC 53.1%. When we focused on those blastocyst with good morphology we had significantly high quality blastocyst in HC; 1.6% vs 9.9%. We also found differences in pregnancy rates between HC and DC, 62.4 vs 55.0% respectively. These differences disappeared in thawed procedures 46.7 % in HC and 50.0% in DC, but the embryos were cultured in normal dry incubator until transfer. In the group of PGT embryos, we had similar embryo ploidy proportional in both groups 39.6%vs 39.2 % in DC
Limitations, reasons for caution: The retrospective nature of this abstract maybe reduce the quality of the evidence presented although sample size is outstanding and embryos were cultured with the type of incubator that included a time-lapse system.
Wider implications of the findings: Culturing embryos with single step media may include high humidity as a preference to optimize pregnancy results.
Trial registration number: none

P-162 Effect of higher donor BMI on clinical pregnancy and live birth rates in IVF cycles.

E. Iovine[1], V. Casciani[1], A. Pristera[1], V. Zazzaro[1], F. Scarselli[1], G. Pirastu[1], D. Uva[1], K. Litwicka[1], A. Greco[1], P.F. Greco[1], M.C. Greco[1], M.T. Varricchio[1], A. Ruberti[1], M.G. Minasi[1], E. Greco[1]

[1]European Hospital, Reproductive medicine, Roma, Italy

Study question: Could higher donor BMI affect the IVF success rate in egg donation cycles?
Summary answer: Increasing donor BMI reduces fertilization rate and embryo development but not clinical outcomes.
What is known already: Excess weight could be deleterious for women causing cardiometabolic health risks and adverse reproductive outcomes. It was known that women with an overweight or obese BMI, experienced worse outcomes in terms of pregnancy and live birth rates that those with normal BMI. It remains unclear whether those effects are exerted at level of the oocyte quality or at the endometrial. The oocyte donor model is a good tool to separate the effect of obesity on oocyte/embryo quality from the effect of endometrial receptivity allowing to study if a higher donor BMI could affect oocytes quality and biological outcomes.
Study design, size, duration: We retrospectively analyzed 85 egg donation cycles (October 2014-January 2020) with ≥ 50% survival rate of vitrified/warmed oocyte. Only cycles with male age ≤ 45 years old were considered. Cycles were divided in two groups: grouP-1 included oocyte-donors with BMI≤23.9 whereas group 2 included donors with BMI≥24. All oocytes were injected with fresh ejaculated normozospermic semen (WHO, 2010). Data, shown as avarage±SD, were analyzed with Chi square or Student-t test.
Participants/materials, setting, methods: GrouP-1 included 57 cycles, donor BMI (21.28±2.76) and 437 oocytes; grouP-2 included 28 cycles, donor BMI (26.03±2.78) and 232 oocytes. Respectively, in grouP-1 and in grouP-2, donor age was 25.39±3.87 and 25.20±3.84, female recipient age was 42.3±3.69 and 41.9±4.18 (NS). The recipient BMI in grouP-1 and in grouP-2 was respectively (22.9±3.80) and (23.1±3.60) (NS), the male average BMI were respectively 25.6±3.43 and 25.8±3.46 (NS). Embryos were culture in a time-lapse incubator until day-3 or day-5.
Main results and the role of chance: Oocytes survival rate in grouP-1 and in grouP-2 was respectively 88.1% (385/437) and 84.9% (197/232)(NS). Fertilization rates in groups 1 and 2 were respectively 77.4% (297/385) and 67.5% (133/197) ($p<0.05$). In grouP-1, a total of 224 embryos (transferred and vitrified) were obtained whereas in grouP-2 total embryos were 91. When the efficiency of treatments was calculated as the ration between total embryos obtained per received oocytes, in grouP-1 a rate was obtained (51.1%, 224/437) higher than in grouP-2 (39.2%, 91/232, $p<0.05$). No statistical differences were found in beta-HCG positive (62.2%, 33/53, in grouP-1; 57.7%, 15/26, in grouP-2), clinical pregnancy rates (CPR) (52.8%, 28/53, in grouP-1; 53.8%, 14/26, in grouP-2) and implantation rate (IR) (40.9%, 35/86, in grouP-1; 45.4%, 20/44, in grouP-2).
Limitations, reasons for caution: In clinical outcomes, only fresh transfers were considered (cumulative data not fully available). Donors was stimulated with protocols according to their history and hormonal levels. The sample size of our population was reduced by selecting male partners <45 year old, a choice made to reduce the possible male-age-factor effect.
Wider implications of the findings: Increasing donor BMI could affect fertilization rate and the amount of embryos obtained suggesting that an ideal BMI value could be set as cut-off in donor screening. Since clinical outcomes were similar in the groups we can hypothesize that endometrial receptivity could adjust the donor BMI effects on oocytes quality.
Trial registration number: not available

P-163 Robust embryo scoring model based on artificial intelligence (AI) applied to a large time-lapse dataset

J. Rimestad[1], M.F. Kragh[2], J.T. Lassen[1], A. Tran[3], J. Berntsen[1]

[1]Vitrolife A/S, Product Development, Viby J, Denmark ;
[2]Vitrolife A/S & Aarhus University, Product Development, Aarhus, Denmark ;
[3]Harrison AI, Co-founder, Sydney, Australia

Study question: Does an AI-based automatic embryo scoring system perform consistently independent of patient age, fertilization method, year of treatment, incubation time, culture media and fertility clinic?
Summary answer: The trained AI model has shown to perform consistently regardless of patient age, fertilization method, year of treatment, incubation time, culture media and fertility clinic.
What is known already: Use of AI for robust selection of embryos requires models trained on large datasets that encompass a wide variation in patient cohorts and clinical practices. Training with a limited dataset would only be directly applicable to the clinical setting where it was developed and would require retraining should clinical practice change.

Previously, an AI model was reported to sort embryos with positive fetal heartbeat (FHB) from all remaining embryos with areas under the receiver operating curves (AUC) from 0.90 to 0.95 (IVY v1.0). However, training AI models can introduce unintentional biases that should be examined using an independent test set.
Study design, size, duration: An AI model was trained for binary classification of positive FHB from all remaining embryos, given time-lapse videos of 98583 embryos from 18 clinics across three continents. The following stratifications were analyzed on a test dataset with 17249 embryos not seen during

training: patient age (for the groups <30, 30-34, 35-39, >39), fertilization method (IVF, ICSI), year of treatment (2011-2019), incubation time (96-115, 115-117, 117-119, 119-130 hours post insemination), culture media and fertility clinic.

Participants/materials, setting, methods: A total of 115832 embryo videos generated by IVF or ICSI between 2011 and 2019 were included in the study. Patient age ranged from 18 to 52. Embryos were cultured using the EmbryoScope or EmbryoScope+ time-lapse system for at least 4 days. 101188 embryos were deemed non-usable due to PGT results or manual deselection by embryologists. 14644 embryos were transferred (fresh or frozen) resulting in 4337 positive FHB and 10307 negative FHB outcomes.

Main results and the role of chance: The AUC on all 17249 embryos in the test dataset was 0.95 [95% CI: 0.943-0.955], showing great overall performance.

For the four age groups, the n and AUC were <30: 2277, 0.97; 30-34: 4302, 0.95; 35-39: 4702, 0.95 and >39: 3857, 0.94. For fertilization methods, the AUC was 0.96 for IVF (n=4681) and 0.95 for ICSI (n=5073). For years of treatment with more than 250 embryos, the mean AUC across all years was 0.95 [0.91 to 0.98]. For the four incubation time groups, the n and AUC were 96-115: 1971, 0.92; 115-117: 3718, 0.94; 117-119: 2579, 0.94; 119-130: 1871, 0.92. For culture media, one clinic using two different brands (n=456 and n=536) was investigated and had AUCs of 0.92 and 0.91. All AUCs above showed only minor biases, indicating generalization across subsets.

Mean AUC across clinics was 0.94 [0.89 to 0.97] for the 12 clinics with more than 250 embryos in the test dataset. To evaluate model generalization to new clinics, leave-one-clinic-out cross validation was performed on these 12 clinics. Each clinic was evaluated on a model trained on the remaining 11 clinics, resulting in a mean AUC of 0.94 [0.91 to 0.97]. The similar AUCs indicate generalization across clinics.

Limitations, reasons for caution: The different clinics from which the data originated did not use a common strategy for embryo selection, which might have introduced biases in model performance. To further validate the AI for embryo selection power, a prospective study will be performed.

Wider implications of the findings: Automatic embryo scoring by AI shows convincing results for all provided subgroups of data and can therefore be expected to generalize well on new clinics, whose data have not been used for training the AI.

Trial registration number: not applicable

P-164 The effect of artificial oocyte activation (AOA) on morphokinetic pattern of human embryos in cases with severe male infertility

M. KARAGIANNI[1], M.I. Papadopoulou[1], C. Oraiopoulou[1], S. Sgoura[1], A. Chatzianastasiou[2], P. Moisidou Tsiorva[2], E. Taki[2], A. Vorniotaki[2], M. Moysidou[1], A. Papatheodorou[1], N. Christoforidis[3], A. Chatziparasidou[4]

[1]Embryolab, Embryology lab, Thessaloniki, Greece ;
[2]Embryolab, Andrology lab, Thessaloniki, Greece ;
[3]Embryolab academy - Embryolab, Clinical, Thessaloniki, Greece ;
[4]Embryolab academy - Embryolab, Embryology lab, Thessaloniki, Greece

Study question: Does artificial oocyte activation (AOA) in cases with severe male infertility alter the morphokinetic pattern of human embryos?

Summary answer: This study suggests that there is no significant difference in morhpokinetics using AOA in cases with 99% abnormal sperm morphology or testicular biopsy.

What is known already: Oocyte activation is the result of a cascade of events triggered by sperm. The moment sperm enters oocyte's cytoplasm it diffuses PLCz which in-turn triggers calcium oscillations followed by oocyte activation and fertilization. In cases were low fertilization rates is observed after ICSI for patients with low sperm count, motility and morphology or patients after testicular sperm extraction (TESE), sperm related activation deficiency may be suspected. To overcome low or no fertilization after ICSI, AOA has been used with varying success rate. At present the available published data is limited and the value of AOA has not been yet determined.

Study design, size, duration: This prospective cohort study included 44 ICSI treatments performed at Embryolab Fertility Clinic, in Thessaloniki, Greece between November 2018 and November 2019. The study population consisted of stimulated cycles with minimum 10 oocytes during oocyte pick up with own or donor oocytes which were fertilized with partner's sperm. The sperm was either from ejaculation with 99% abnormal morphology or from testicular biopsy.

Participants/materials, setting, methods: 44 cycles were analyzed in order to evaluate their morphokinetic pattern. Mature oocytes from each patient were divided in two groups: AOA group included oocytes that were incubated in Ca^{2+} ionophore (A23187, CultActive, Gynemed) after ICSI for 15 minutes and NO AOA group was the control. All oocytes were cultured in Embryoscope time-lapse incubator and the key time parameters and dynamic events were analyzed using t-test and Mann–Whitney U test.

Main results and the role of chance: There was no significant difference in fertilization rate between 2 groups (AOA-68.41% vs. NO AOA-65.88%, p>0.05). Furthermore, blastocyst rate was similar between the 2 groups (AOA-61.48% vs NO AOA-65.74%, p>0.05). The comparison for key time parameters and dynamic events (tPB2, tPNa, tPNf, t2, t3,t4, t5, t6, t7, t8, t9+, tSC, tM, tSB, tB, tEB, tHB, S2, S3, S5, CC2, CC3, Blastulation) showed no significant difference between the AOA group and NO AOA group in total (p>0.05). In addition, when AOA groups were divided into 2 subgroups a) ejaculated sperm with 99% abnormal morphology and b) testicular sperm, the morphokinetic comparison of these 2 separated groups to their control did not reach any significant differences (p>0.05).

Limitations, reasons for caution: Only a limited number of cases were included in this study. Apart from severe male infertility other maternal derived factors may also be involved. The results for pregnancy, clinical pregnancy and live birth were not available for all cases.

Wider implications of the findings: Artificial oocyte activation method does not seem to have an effect on the morphokinetic pattern and embryonic development. Additionally, a benefit on fertilization rate in cases of ejaculated sperm with 99% abnormal morphology or testicular sperm was not observed.

Trial registration number: N/A

P-165 KIDScore helps in selecting the best euploid embryo: a retrospective cohort study

L. Pellegrini[1], L. Parmegiani[1], A. Rastellini[1], A. Arnone[1], S. Bernardi[1], S. Lanzilotti[1], A.M. Maccarini[1], E. Troilo[1], G.E. Cognigni[1], M. Filicori[1]

[1]GynePro Medical Centers- NextClinics International, Reproductive Medicine Unit, Bologna, Italy

Study question: Does KIDScore D5v2 represent an additional tool for the selection of euploid blastocysts and for predicting their implantation?

Summary answer: KIDScore D5v2 appears to be predictive to discriminate between euploid and aneuploid embryos and euploid blastocysts with KIDScore 6.0-10.0 show a significantly higher implantation rate.

What is known already: Time-lapse microscopy (TLM) offers continuous monitoring of embryo development and KIDScore is the software used by the TLM incubator Embryoscope (Vitrolife, Sweden) to score embryo morphokinetics, but the prediction of chromosomal abnormalities using morphokinetics alone is still insufficient. Preimplantation Genetic Testing for Aneuploidy (PGT-A) discriminates between euploid and aneuploid blastocysts; KIDScore could be used to prioritize the transfer of euploid embryos identified by PGT-A diagnosis.

Study design, size, duration: Retrospective cohort study, from June 2016 to December 2019 on 508 embryos from 156 patients, cultured to blastocyst stage (DAY 5-7) and screened by PGT-A. Based on KIDScore, blastocysts were divided in two groups: Group 1 (KS 0 – 5.9; n=320), Group 2 (6.0 – 10; n=188) Main outcome measures: euploidy rate (number of euploid blastocysts/total blastocysts), implantation rate (number intrauterine gestational sacs observed by transvaginal ultrasonography/n° of transferred blastocysts).

Participants/materials, setting, methods: Mean female age ±SD was 36.0±4.4 (25-46). Blastocysts were cultured in Embryoscope, assessed by KIDScore model D5v2 and biopsied to perform PGT-A (Next Generation Sequencing Veriseq PGT protocol - Illumina, USA). Subsequently, blastocysts were vitrified and euploid blastocysts were warmed and transferred (100 blastocysts; mean 1.14± 0.35).

Main results and the role of chance: Female and male mean age was comparable between the groups. Euploidy rate was statistically different: 24.6% (79/320) in Group 1 (KS 0 – 5.9) versus 36.7% (69/188) in Group 2

(KS 6.0 – 10; P=0.0040 Chi-squared test), suggesting KIDScore seems to be predictive for selecting euploid blastocysts. Implantation rate was calculated over the 100 blastocysts transferred; the mean number of transferred embryos was comparable in each group (Group 1: 1.13±0.34; Group 2: 1.16±0.37). Implantation rate was significantly lower at 28.2% (13/46) in Group 1 compared to 51.8% (28/54) in Group 2 (P=0.0288 Yates's Chi-squared test). This result suggests a threshold value based on morphokinetic features that could help embryologists to prioritize euploid blastocysts prior to transfer. Selecting euploid blastocysts with KIDScore > 6 seems to promote increased chances of implantation.

Limitations, reasons for caution: This study is limited to patients from only one centre with a relatively low number of blastocysts. Further and larger studies are needed to validate these results and to permit efficient clinical implications.

Wider implications of the findings: Blastocyst selection through time-lapse technology alone should not be considered as a replacement for PGT. According to our findings, KIDScore seems to be a potentially valuable tool for identifying the euploid blastocysts having a higher probability of implantation.

Trial registration number: N/A

P-166 The impact of incubator humidity, protein supplementation and time-lapse dish design on media osmolality during culture.

M. Garcia-Jimenez[1], E. Mestres[1], A. Casals[1], I. Vanrell[1], A. Villamar[1], M. Acacio[1], Q. Matia[1], J. Cohen[2], N. Costa-Borges[1], G. Calderon[1]

[1]Embryotools S.L., Research & development, Barcelona, Spain ;
[2]ART Institute of Washington, IVF Laboratory, New York- NY, U.S.A.

Study question: How do incubator humidity, protein supplementation and time-lapse dish design affect the osmolality of culture media over time?

Summary answer: Incubator humidity and time-lapse dish design play an important role in the stability of osmolality during culture. Protein supplementation does not alter osmolality over time.

What is known already: Uninterrupted embryo culture is a widespread practice, but certain key conditions must be considered to avoid osmolality increasing above detrimental thresholds. Humidified incubation may protect against evaporation, but humidity is a non-binary condition and can vary between incubators.

Proteins (like albumin) can have emulsifying properties and may have a potential effect on osmolality during culture under oil. Time-lapse systems are usually dry incubators and embryos are cultured uninterruptedly. In addition, the preparation of time-lapse dishes is time-consuming and uses reduced volumes of media and oil, which may result in some evaporation.

Study design, size, duration: Medium osmolality was measured daily between 0-168h in Petri dishes with 20µl drops overlaid with oil, comparing incubation in a humid *vs.* dry atmosphere and media with 0 *vs.* 20mg/ml HSA. Osmolality was also measured in six different types of time-lapse dishes after 0h (set-up), 72h (Day 3), 120h (Day 5) and 168h of culture (Day 7). All readings were performed with a last-generation vapour pressure osmometer (VAPRO 5600, Wescor).

Participants/materials, setting, methods: For the humid *vs.* dry and protein *vs.* no-protein experiments, 35mm Petri dishes were prepared following an underlay method by placing the medium droplets below the oil to avoid any evaporation during setup. Each type of time-lapse dish was prepared according to its manufacturer's instructions. All six types of time-lapse dishes were cultured in a joint dry benchtop incubator. For two of those models, culture and measurements were replicated in their time-lapse incubator.

Main results and the role of chance: Results are shown as the difference between the initial osmolality (once the dish was set-up) and after 120/168h of culture. When assessing the effect of protein supplementation, osmolality increased by 28/41 and 30/45 mOsm/kg in the 0 and 20 mg/ml HSA groups, respectively. In a humid atmosphere, a rise of 15/19 mOsm/kg was observed, compared with 34/42 mOsm/kg in a dry incubator.

The set-up of all the studied time-lapse dishes lead to medium evaporation by 5 mOsm/kg in relation to the medium vial. An additional increase of osmolality was detected during culture, notably varying depending on the dish design. For the six studied dish models, the observed increases in a dry benchtop incubator were: 12/20, 16/20, 15/22, 22/30, 27/35 and 31/42 mOsm/kg. Two dish types could be cultured in their corresponding time-lapse dry incubators, as well. In these cases, the osmolality increase was comparable to that observed in the dry benchtop suggesting that a direct dish fit in the heated plate within time-lapse incubators does not significantly increase evaporation.

Limitations, reasons for caution: The humidity level in benchtop incubators may vary and is not usually monitored. In this study, time-lapse dishes were cultured in a dry benchtop incubator. However, some time-lapse incubators allow for a humidified atmosphere, and its effects on osmolality must be studied in each specific dish incubator.

Wider implications of the findings: The starting osmolality of culture media, incubator humidity and time-lapse dish design are important factors to consider during *in vitro* embryo culture. An excessive evaporation during uninterrupted culture will result in osmolality increase and may have a detrimental effect on embryo development.

Trial registration number: N/A

P-167 Antagomir-Mediated Silencing of let-7a-5p in Mouse Embryo can improve blastocyst Implantation Rate.

J. Shakerzadeh[1], M. Movahedin[2], A. Eidi[1], N. Hayati Roudbari[1], K. Parivar[1]

[1]Science and Research Branch- Islamic Azad University, Department of Biology, Tehran, Iran ;
[2]Faculty of Medical Sciences- Tarbiat Modares University- Tehran- Iran., Department of Anatomical Sciences, Tehran, Iran

Study question: To evaluate the role of murine let-7a-5p blastocysts in implantation failure and the possibility of let-7a-5p suppression in improvement of implantation *in vivo.*

Summary answer: Our results suggested that suppression of embryonic murine let-7a-5p could improve embryonic implantation through the upregulation of Igf1, Itgb3 and Tgfb1.

What is known already: Blastocyst implantation needs appropriate molecular and cellular communications between blastocyst. microRNAs-mediated gene expression of both endometrial tissues and/or blastocyst have impact in the process of implantation and fetal development.

Study design, size, duration: This is a case-control study and totally 100 adult female mice and 10 adult male mice during 2018-2019 were included (Strain NMRI). We analyzed the expression of murine let-7a-5p in the control, sham, and treatment embryos (8-cells stage) by quantitative reverse transcription PCR (qRT-PCR).

Participants/materials, setting, methods: In the treatment group, IVF drops were transfected with mmu-let-7a-5p antagomir (ABM, Inc., Richmond, BC, Canada) using ViaFect™ Transfection Reagent (Promega, Madison, WI, USA), while the sham group receive only transfection reagent, and control group receive no treatment. Expression levels of mRNA targets of let-7a-5p (Igf1, Il1, Itgb3 and Tgfb1) were analyzed by qRT-PCR. The blastocysts were transfer to the pseudopregnant mice to evaluate the effect of let-7a-5p supression on embryo implantation.

Main results and the role of chance: : Our results showed that there were not significant changes in the developed embryo to the blastocyst stage between the study groups (P> 0.05). Expressions level of let-7a-5p in the treatment group were significantly downregulated compared with the controls (FC=0.352, P=0.039). In contrast, no significant changes were observed between the sham and control groups (P> 0.05). Expression level of the four mRNA targets was not significantly different between the sham and control group (P> 0.05). Expression level of Igf1 (FC=1.8, 0.021), Itgb3 (FC=1.3. P=0.039) and Tgfb1 (FC=1.4, P=0.032) were significantly upregulated in the anatgomir treated group compared to the control group. The implantation rates were significantly higher in the treatment group compared to the control (P< 0.05).

Limitations, reasons for caution: Since each microRNA can target many of transcripts, we should explore the other potential mRNAs of let-7a-5p in the process of implantation.

Wider implications of the findings: A higher implantation rate following the suppression of murine let-7a-5p embryo suggested that microRNAs have important roles in the process of implantation.

Trial registration number: not applicable

P-168 A prospective study of the effect of laser-assisted hatching on the clinical outcome of single frozen/thawed embryos transfer cycles

V. Torres Monserrat[1], M.C. Delpiccolo[1], M.N. Lardizabal[1], N. Carizza[2], T. Leiva[2], E. Navall[2], S. Dominici[2], R. Sol Ariadna[2], C. Carizza[3]

[1]Fertya-Grupo Oroño, Embryology Laboratory, Rosario, Argentina ;
[2]Fertya-Grupo Oroño, Gynecology, Rosario, Argentina ;
[3]Fertya-Grupo Oroño, Medical Director, Rosario, Argentina

Study question: Does laser-assisted hatching (AH) improve clinical pregnancy outcome of frozen/thawed embryo transfer cycles taking into consideration the day of embryo development and patients´ prognosis?
Summary answer: Laser-assisted hatching does not improve clinical pregnancy rate in frozen/thawed embryo transfer. However, good prognosis patients that transfer single day-5 embryos are affected with AH.
What is known already: Although assisted hatching (AH) is routinely performed in frozen/thawed embryo transfer cycles, there is not enough data to conclude the benefit of this technique. Despite its widespread use, there is a limited number of prospective studies that have examined the effect of AH on clinical pregnancy and multiple pregnancy. Most of the investigations with good scientific evidence conclude that AH can benefit clinical pregnancy rate in patients with poor prognosis, but it could also increase multiple pregnancy. However, there are not studies that evaluate the effect of AH and the day of embryo development in frozen/thawed embryo transfer
Study design, size, duration: A prospective analysis of the effect of laser-assisted hatching on clinical and multiple pregnancy rates in 331 patients that underwent frozen/thawed single embryo transfer in our clinic from April to December 2019. Before treatment, patients were explained about actual evidence of the technique for them to decide to do it or not. We compare Control Group, 120 transfer without AH, and Treatment Group, 211 patients with AH. Embryo development day and patient's prognosis were analysed
Participants/materials, setting, methods: Embryos were incubated in K-System® G-185 incubator with Sage® Medium. Kitazato® Medium Kits were used to vitrify and thaw. Single embryo transfer was performed the same day embryo was thawed. AH was performed immediately after thawing with Lykos® laser set in the multipulse mode (pulse duration 200us). The chi square independence test and Fisher's exact test were used to compare groups about clinical pregnancy and multiple pregnancies rates, significance represented by a $p<0.05$
Main results and the role of chance: Laser-assisted hatching does not improve clinical pregnancy rate (Control Group: 31% vs Treatment Group: 27%, P-value=0.404) nor affect multiple pregnancy rate (Control Group: 5.4% vs Treatment Group: 1.8%, P-value=0.561) when considering all patients undergoing frozen/thawed single embryo transfer. When analysing only patients with bad prognosis, Control Group: 9% vs Treatment Group: 20%, P-value=0.083, this technique does not improve clinical results either. The same result was obtained when studying patients with good prognosis, Control Group: 45% vs Treatment Group: 33%, P-value=0.088. In addition, we analysed the same groups and divided them considering the day of embryo development when vitrified. When considering all patients undergoing frozen/thawed day-5 embryo transfer, clinical pregnancy was not benefited by AH (Control Group: 39% vs Treatment Group: 28%, P-value=0.125). However, when this sub-group was divided according to prognosis, good prognosis patients were affected by laser-assisted hatching (Control Group: 53% vs Treatment Group: 32%, P-value=0.021), whereas, bad prognosis patients were not affected (Control Group: 13% vs Treatment Group: 24%, P-value=0.257). Surprisingly, these results were not seen in the sub-group of day-6 embryo transfer where clinical pregnancy is not affected by performing AH in the general population (P-value=0.617), nor in good prognosis (P-value=0.759) or bad prognosis (P-value=0.255) patients
Limitations, reasons for caution: The main limitation of this study is the lack of randomness between control group and treatment group
Wider implications of the findings: This prospective study shows that assisted hatching does not benefit clinical pregnancy rates of frozen/thawed single embryo transfer cycles. This is the first prospective study that analyse the effect of AH on frozen/thawed single embryo transfer in relation to embryo development day and patients prognosis.
Trial registration number: -

P-169 Early recombinant-LH supplementation improves oocyte competence acting on oxidative stress in young poor responder IVF-patients.

M. Santonastaso[1], F. Caprio[2], A. Natella[2], F. Mottola[3], L. Rocco[3], R. Ianniello[2], D. Mele[2], N. Colacurci[1]

[1]Università degli Studi della Campania L. Vanvitelli, Dpt. of Woman-Child and General and Special Surgery, Naples, Italy ;
[2]Azienda Ospedaliera Universitaria L. Vanvitelli, Gynecological-obstetric clinic, Naples, Italy ;
[3]Università degli Studi della Campania L. Vanvitelli, Dpt. of Environmental-Biological and Pharmaceutical Sciences and Technologies, Caserta, Italy

Study question: Does early recombinant (rec)-LH supplementation in ovarian stimulation protocol affect oxidative stress in cumulus cells as well as IVF outcome in poor responder patients?
Summary answer: Early rec-LH supplementation enhances ovarian response to stimulation and embryological parameters reducing oxidative stress markers in young women with poor ovarian reserve in ICSI cycles.
What is known already: Management of women with poor ovarian response (POR) to stimulation is a major challenge in reproductive medicine. The POR primary causes remain unknown and oxidative stress was recommended as one of causes. Multiple interventions have been proposed to improve reproductive outcomes in POR but showed conflicting results. Ovarian stimulation may have a direct impact on oxidative stress markers with Reactive Oxygen Species (ROS) production and perturbation of oxidant-antioxidant balance. IVF outcome is adversely affected if imbalance exists between ROS and antioxidants in oocyte microenvironment. Early rec-LH supplementation impact in POR ovarian stimulation protocol to improve IVF outcome are still unclear.
Study design, size, duration: This prospective randomized controlled study included 132 POR patients stratified according to POSEIDON classification group 3(age < 35, poor ovarian reserve parameters)from March 2015 to April 2018. All patients enrolled underwent controlled ovarian stimulation according to GnRH antagonist protocol. Patients were randomized to rec-LH supplementation(A group) or no rec-LH supplementation(B group) from second day of the cycle. Oxidative stress markers in cumulus cells,oocyte number and quality,fertilization rate, embryo quality, pregnancy rates are the outcome measures.
Participants/materials, setting, methods: Exclusion criteria: PCOs, endometriosis,metabolic and endocrinology diseases, severe oligoasthenoteratozoospermia. On the day of oocyte retrieval, cumulus cell samples were mechanically removed from oocyte. The intracellular ROS were assessed by H2DCF-DA (2',7'-dichlorofluorescein diacetate) fluorescent probe. DNA fragmentation index (DFI) and apoptotic gene (BAX and BCL-2) expression was assessed by TUNEL Test and qPCR-Real Time respectively. The data were analyzed using the unpaired Student's t-test by IBM SPSS-STATISTICS versions 20.0 and considered significant if P-value ≤ 0.05.
Main results and the role of chance: There was no significant difference in the baseline clinical characteristics and the number of oocyte retrieval in both groups. The mature oocytes number and number of fertilized oocytes were higher in patients treated with rec-FSH + rec-LH (A group). The average number of high-quality embryos on day 3 in patients treated with rec-FSH + rec-LH and rec-FSH was 1.70±0.75 and 0.66±0.47 respectively ($p\leq 0.05$). After the two ovarian stimulation protocols the differences of number of transferred embryos was statistically significant. The pregnancy rate was statistically higher in patients treated with rec-FSH + rec-LHcompared to patients treated with rec-FSH (59.37% versus 34.30%, $p\leq 0.05$). Intracellular ROS percentage was significantly lower in POR patient treated with rec-FSH plus rec-LH compared POR patients no rec-LH supplementation (23±5.80 vs. 43±7.50, $p\leq 0.05$). Cumulus cell DNA fragmentation was significantly lower in in patients treated with rec-FSH + rec-LH. Cumulus cells surrounding oocytes retrieved from patient treated with rec-FSH + rec-LH expressed significantly higher BCL-2 mRNA levels than those from patient treated with rec-FSH ($p\leq 0.05$) whereas BAX mRNA levels resulted to decrease significantly ($p\leq 0.05$) in the patients treated with rec-FSH + rec-LH compared to patients treated with r-FSH.
Limitations, reasons for caution: Lifestyle factors (i.e. smoking, diet) are not considered. The cumulus cell samples are pooled.
Wider implications of the findings: This study showed that rec-LH supplementation reduces oxidative stress markers in term of intracellular ROS

production, DNA fragmentation and apoptosis as well as influences oocyte competence in POR patients. Early rec-LH supplementation in controlled ovarian stimulation protocol act for strategy to improve IVF reproductive outcomes in young POR patients.

Trial registration number: N° 48 (30/01/2015)

P-170 Non-invasive detection of metabolically impaired euploid blastocysts with low implantation potential

S. Cabello-Pinedo[1], H. Abdulla[2], M.L. Seth-Smith[1], M. Escriba[3], J. Crespo[3], J.A. Horcajadas[1], S. Munne[1]

[1]Overture Life SL, Scientific Department, Madrid, Spain ;
[2]Texas A&M University Corpus Christi, Chemistry, Corpus Christi, U.S.A. ;
[3]Juana Crespo Clinic, Embryology, Valencia, Spain

Study question: As not all euploid embryos implant, is it possible to identify non-implanting euploid blastocysts from their spent media metabolomics profile?

Summary answer: Analysis of the spent media metabolomics profile could predict about 30% implantation failure of euploid embryos, coinciding with the upper limit of PGT-A success.

What is known already: Embryo implantation potential and euploidy rates decrease with advancing maternal age. Preimplantation Genetic Testing (PGT) is been used in routine clinical practice to avoid the transfer of aneuploid embryos with minimal chance of resulting in a viable pregnancy. However, even the best PGT-A published results do not exceed 70% ongoing pregnancy rates. Little is understood of the causes of failure of euploid embryos to implant, and methods to discriminate between viable euploid embryos and non-viable euploid embryos, that will not result in a successful pregnancy, have not been developed yet.

Study design, size, duration: This study includes spent media samples collected before trophectoderm biopsy from embryos that were later analyzed by PGT and transferred. To develop the algorithm, this study used a dataset that included 37 samples collected using Vitrolife media that were classified as pregnancy (P) or non-pregnancy (NP) according to embryo implantation outcome after transfer. The algorithm was then tested on spent media of 42 euploid blastocysts.

Participants/materials, setting, methods: For the training subset (n=37), patients undergoing infertility treatments were included. For the validation stage (n=42), patients whose embryos were analyzed through PGT were recruited. In both cases, spent media samples (20-40ml) were collected after incubation between days 3 and 5 and just before biopsy. Before the analysis, samples were diluted with distilled water, ultrafiltered to remove molecules >3KDa and run through a UPLC-Fusion Orbitrap MS/MS system to determine metabolite concentration.

Main results and the role of chance: Samples from the training subset were processed, detecting more than 5,550 metabolites in spent media samples. Several statistical techniques were applied to reduce this large number of metabolites to the most informative ones for pregnancy outcome. This led to the definition of a Vitrolife specific MPI (Metabolite Pregnancy Index), which has proved to be highly predictive of both P and NP embryos. This technique was tested in a completely different data subset showing an ability to identify 78% of P samples and 61% of NP samples.

For the validation part of the study, metabolic profile of culture media coming from euploid embryos (n=42) was studied in order to assess whether the implantation failure of some euploid embryos may be due to metabolic impairment. A 30.95% of euploid embryos in the validation subset showed a metabolomic profile predicting poor implantation potential despite being euploid.

Although further research is necessary, these results open the possibility of applying metabolomics to differentiate, within euploid embryos, those that are viable from the ones that will not result in a successful pregnancy.

Limitations, reasons for caution: The study was retrospective but a prospective clinical trial is underway. In addition, all the samples were collected using the same culture media and the metabolomic algorithm is media specific. The study needs to be validated in multiple culture media and test its outcome comparing it with more clinical data.

Wider implications of the findings: This study represents an initial step in the development of non-invasive methods based on metabolomics approach, that allow identification of viable embryos, not only based on ploidy status. The optimization of embryo selection techniques, without altering embryology protocols, may result in an improvement in pregnancy outcomes in women undergoing ART.

Trial registration number: NA

P-171 Is the frequency of aneuploidy and mosaicism at blastocyst stage associated to the embryo sex?

P. Villanueva[1], J. Huayhua[2], L. Noriega-Hoces[3], F. Vizcarra[1], J. Noriega-Portella[3], L. Guzman Masias[1]

[1]Laboratorios PRANOR, Embriologia, Lima, Peru ;
[2]ADN Diagnostico, Genetics, Lima, Peru ;
[3]Clinica Concebir, Departamento Medico, Lima, Peru

Study question: Is the chromosome constitution associated to the embryo sex?

Summary answer: Male blastocysts are less likely to be euploid considering confounding variables. Additionally, they have a higher chromosomal unbalances (aneuploidy+mosaicism) in the same embryo.

What is known already: There is very limited information about embryo euploidy and mosaicism in relation to embryo sex. In our study, chromosome constitution at blastocyst stage was evaluated by next-generation sequencing (NGS) and confounding factors were controlled. Previous studies with lower data found that embryo sex was not significantly associated with ploidy status.

Study design, size, duration: Data was collected retrospectively from June 2016 to October 2019. In total, 1212 PGT-A cycles were included.

Participants/materials, setting, methods: In total, 4434 blastocysts analysed with PGT-A were generated from 1212 cycles. The chromosome constitution was classified: euploid, single-aneuploid, double-aneuploid, complex-aneuploid, single mosaic, double mosaic, complex mosaic, mix (aneuploid and mosaic) and complex mix.

A multilevel model was made and associations between variables by logistic regression adjusted according to maternal age, blastocyst grade, biopsy day, inner cell mass and trophectoderm grade, fertilization method, and embryologist.

Main results and the role of chance: The mean maternal age was 36.2 ± 4.2. The genetic results of 4434 blastocyst were as follow: euploid 46.4% (N= 2058); single aneuploid 14.4% (N=640); double aneuploid 4.7% (N= 208), complex aneuploid 2.1% (N=94); single mosaic 13.7% (N=607), double mosaic 4.7% (N=210); complex mosaic 6.6% (N=291); mix 2.8% (N=123) and complex mix 4.6%(N=203). When results were classified according to the embryo sex, 49.1% (N=1010) were euploid female blastocyst and 50.9% (N=1048) were male euploid blastocyst. For single aneuploidy was 47.7% (N=305) and 52.3% (N=335); double aneuploid was 46.2% (N=96) and 53.8% (N=112); complex aneuploidy was 46.8% (N=44) and 53.2% (N=50); single mosaic was 44.3% (N=269) and 55.7% (N=338); double mosaic was 52.9% (N=111) and 47.1% (N=99); complex mosaic was 46% (N=134) and 54% (N=157) and complex mix 47.8% (N=97) and 52.2% (N=106) in female and male blastocyst respectively (p≥0.05). Male embryos are more likely to have a mix chromosome constitution (aneuploid and mosaic) than female embryos (OR=1.63, 95% CI (1.12-2.36), p=0.010). Furthermore, they are less likely to be euploid (OR=0.88, 95% CI (0.78-0.99), p=0.037) adjusted for all variables.

Limitations, reasons for caution: The results observed in this study should be confirmed using a larger number of samples.

Wider implications of the findings: Our data suggest that male blastocyst are associated with a lower euploidy and a higher mixture of chromosomal unbalanced.

Trial registration number: none

P-172 The transcriptome of paired human oocytes related to progesterone primed ovarian stimulation

F. Martinez[1], S. Mateo[1], E. Clua[1], N. Coca[2], J. Rodriguez-Lumbiarres[2], L. Armengol[2], M. Roca[1], S. Garcia[1], N. Polyzos[1]

[1]Hospital Universitari Dexeus, Obstetrics- Gynecology and Reproduction, Barcelona, Spain ;
[2]qGenomics, qGenomics, Barcelona, Spain

Study question: Is there any difference in the human oocyte transcriptome under two specific Controlled Ovarian Stimulation (COS)/LH suppression protocols: progesterone-primed cycles (PP) and GnRH-antagonist cycles (ANT).
Summary answer: Different modes of controlling the endogenous LH secretion do not translate in a significantly different oocyte transcriptome.
What is known already: Although the use of and oral progesterone (PP) instead of (ANT) has been effective in controlling LH surge during ovarian stimulation, evidence from a randomized trial reported lower live birth rates from PP-derived oocytes, suggesting a detrimental effect on oocyte competence. A novel potential method to assess an effect of stimulation on the oocyte level is single-cell transcriptome, though powerful genomic techniques allow this even in a single-cell. In the current study we aimed to evaluate the effect of PP stimulation on oocyte developmental competence, through transcriptome analysis of In-vivo-matured oocytes (transcriptome remarkably stable across ages and ovarian reserve).
Study design, size, duration: Retrospective study comparing single-cell transcriptome of 24 mature paired oocytes from four oocyte donors (OD) who underwent two consecutive COS/IVF cycles at private, university-based IVF center between January 2017 and March 2018 were included.

OD underwent two COS/IVF cycles, under two LH suppression protocols (PP-Group: 75mg daily oral Desogestrel; ANT-Group: 0.25mg daily s.c. Ganirelix) until GnRH agonist trigger.
Participants/materials, setting, methods: Four healthy oocyte donors underwent two consecutive COS/IVF cycles, under two LH suppression protocols (PP-Group: 75mg/day Desogestrel; ANT-Group: 0.25mg/day Ganirelix) until trigger. Mature MII oocytes were vitrified according to standard procedure. Three oocytes from each cycle (PP/antagonist) from the same donor were thawed, total-RNA was extracted, cDNA was prepared and labeled prior to hybridization on PrimeView Human Gene Expression arrays(Affymetrix). Gene expression levels were normalized (RMA) and compared between treatments after data normalization (LIMMA).
Main results and the role of chance: Overall, we analyzed the transcriptome from 24 oocytes derived from 4 oocytes donors who underwent 2 consecutive ovarian stimulation cycles, 1 with GnRH antagonist and 1 with PP (12 oocytes from ANT and 12 oocytes from PP).

Comparisons in the gene expression profile from total single-cell RNA extracted from oocytes derived from ANT or PP ovarian stimulation did not demonstrate any significant difference. Following, adjusting P-values for multiple testing, no statistically significant differences in gene expression levels were observed between oocytes obtained from the two evaluated Controlled Ovarian Stimulation (COS)/LH suppression protocols. Despite that single-cell transcriptome profile did not differ when comparing different LH suppression protocols (ANT vs. PP), of interest, other experimental conditions have been have significantly influenced oocyte transcriptome (P<0.05).
Limitations, reasons for caution: The study was restricted to healthy OD and thereby other conditions related to infertility or older age were not considered. This is a descriptive study with a limited number of samples reflecting the difficulty to recruit human oocytes, especially from women with infertility.
Wider implications of the findings: To our knowledge this is the first study utilizing singe-cell transcriptome analysis in order to evaluate the effect PP suppression of LH on oocyte developmental competence. Our results add reassuring information to the clinical available data regarding the safety of the PP ovarian stimulation protocols
Trial registration number: Not Applicable

P-173 Interest of laser assisted hatching prior to good-quality blastocyst transfer: intermediate results of a prospective randomized study

C. Herbemont[1], Y. Boumerdassi[1], B. Bennani Smires[1], S. Sarandi[1], O. Selmi[1], C. Vinolas[2], C. Lethielleux[2], F. Krief[2], I. Cedrin-Durnerin[2], M. Peigné[2], M. Grynberg[2], C. Sifer[1]

[1]Hôpital Jean Verdier, Histologie-Embryologie-Cytogénétique-Biologie de la Reproduction-CECOS, Bondy, France ;
[2]Hôpital Jean Verdier, Médecine de la Reproduction, Bondy, France

Study question: The aim was to assess the impact of laser assisted hatching (AH) on the implantation potential of good-quality blastocysts (GQB) transferred in a selected population.
Summary answer: In good-prognosis patients and those with recurrent implantation failure, implantation rates (IR) of GQB were similar whether laser-AH was performed or not before transfer.
What is known already: In assisted reproductive techniques, embryo development conditions are deeply modified. Indeed, the increasing use of extended culture and vitrification may induce a zona hardening, possibly resulting in a defective hatching process and implantation failure. Assisted Hatching, defined as the artificial breakage of the zona pellucida (ZP), appears to be a tool to overcome this issue and improve IVF/ICSI outcomes. To date, several studies have focused on the outcomes of AH performed at the cleavage-stage, concluding to an increase in clinical pregnancy rates, especially in poor-prognosis patients. However, investigations on the interest of AH at the blastocyst stage are still controversial.
Study design, size, duration: This prospective randomized study has been in progress since September 2017. Inclusion criteria were: female age ≤37 years, fresh/vitrified day-5 GQB (defined as: blastocoel expansion B3/B4, inner cell mass and/or trophectoderm graded A/B, according to the grading system described by Gardner and Schoolcraft), absence of risk factors for implantation failure. So far, 260 fresh/vitrified GQB transfers have been randomly allocated to AH group (n=122): laser-AH performed prior to transfer; or no-AH group (n=138): no hatching.
Participants/materials, setting, methods: In AH group, a single hole completely through the ZP was performed using a 200-µsecond laser pulse (Lykos, Hamilton Thorne).

In the preliminary analysis, the included population was stratified by number of good-quality (GQ) embryos previously transferred, leading to "good prognosis" (GP) and "recurrent implantation failure" (RIF) subgroups (respectively defined as ≤4 and >4 GQ cleavage-stage embryos, or ≤2 and >2 GQB transferred without proven implantation). Clinical outcomes were compared between AH and no-AH groups.
Main results and the role of chance: In the "GP" analysis, AH (n=108) and no-AH (n=117) groups were comparable considering female age, body mass index, type and cause of infertility, and attempt rank. On average, 1,13±0,34 and 1,08±0,28 GQB were transferred in AH and no-AH groups, respectively (p=0,29). Quality of the transferred blastocysts was similar as well: 57,4% of toP-blastocysts [B4AA/AB/BA] in AH group vs. 59,1% in no-AH group, respectively (p=0,60). IR and clinical pregnancy rates (CPR) were not significantly different whether laser-AH was performed or not prior to transfer (IR: 39,4% in AH group vs. 43,6% in no-AH group; p=0,51, and CPR: 40,7% vs. 45,3%, respectively; p=0,17).

Considering the "RIF" subgroups, patients' characteristics and the mean number of GQB transferred (1,57±0,51 vs. 1,46±0,51, respectively; p=0,51) were similar between AH (n=13) and no-AH (n=23) groups. There was no difference in the proportion of toP-blastocysts transferred (66,7% vs. 69,7%, p=0,82). Interestingly, GQB transferred after laser-AH yielded similar IR and CPR compared to GQB transferred without laser-AH (IR: 42,9% in the two groups; CPR: 50,0% vs. 54,5%, p=0,79).
Limitations, reasons for caution: These preliminary results need to be confirmed on a larger series, especially in the "RIF" subgroup. Moreover, a potential impact of AH on twin pregnancy rates should be examined.
Wider implications of the findings: If confirmed, these findings would suggest that assisted hatching might not be beneficial to young patients, with or without history of implantation failure, as long as good-quality blastocysts are transferred. Finally, the hypothesis of zona hardening due to exposure to culture and/or vitrification media might be questioned.
Trial registration number: 2018-A02395-50

P-174 The effect of ovine whole ovary cryopreservation on the antral follicle oocyte and its developmental potential

A. Dadson[1], D. Wang[1], J. Hernandez-Medrano[1], W. Maalouf[1]

[1]University of Nottingham, Department of Obstetrics and Gynaecology, Nottingham, United Kingdom

Study question: Are oocytes obtained from antral follicles of sheep viable with the capacity to mature following whole ovarian cryopreservation (WOCP) using the slow freezing method?
Summary answer: Oocytes isolated from whole ovary cryopreserved and thawed antral follicles fail to mature *in vitro* and record high level of induced apoptosis.

What is known already: Whole ovary cryopreservation using slow freezing and transplantation restores ovarian function and natural fertility resulting in multiple live births in sheep. This is as a result of the preservation and survival of primordial and preantral follicles.
Study design, size, duration: A total of 540 antral follicle oocytes (control, CT, n=304 and WOCP, n=236) were aspirated from abattoir-sourced sheep ovaries. After collection, oocytes were classified according to cumulus oocyte complex (COC) morphology into normal (A&B) or abnormal (C&D). Oocytes were matured *in vitro* and nuclear maturation assessed after 24 hours. The viability and apoptosis of antral follicle oocytes was also assessed immediately following cryopreservation and subsequent thawing.
Participants/materials, setting, methods: Ovaries were randomly allocated into fresh (control, CT) and WOCP. WOCP and thawing was as described by Onions et al 2008. COCs were then retrieved and grouped into the various grades based on the cytoplasm and presence or absence of cumulus cells. The oocytes were then assessed for viability and apoptosis using propodium iodide (PI) and terminal deoxynucleotidyl fluorescein-dUTP nick end labelling (TUNEL) respectively. Nuclear maturation was assessed by DAPI following *in vitro* maturation (IVM).
Main results and the role of chance: Percentage of COCs classified as normal was similar (P>0.05) between WOCP (54.5±12.7%) and CT (54.3±8.0%). Moreover, PI positive oocyte percentages were comparable (P>0.05) between WOCP and CT: 32.6% (n=23/70) and 19.8% (n=15/76), respectively. However, nuclear maturation was lower (p<0.05) in WOCP (8%, n=1/64) compared to CT oocytes (67%, n=53/65), whereas TUNEL apoptotic index was higher in WOCP oocytes than controls (75.4%, n=52/69 vs. 26.6%, n=17/76).
Limitations, reasons for caution: Whole ovary cryopreservation caused a significant loss of cumulus cells in the oocytes obtained from antral follicles. These results depict clearly shows the inability of the oocytes to develop further, however does not conclude on the quality of the oocyte.
Wider implications of the findings: Isolating oocytes from antral follicles of freeze-thawed sheep ovary for *in vitro* maturation and fertilisation may not be a feasible path for clinical application.
Trial registration number: not applicable

P-175 The impact of anti-centromere antibodies (ACAs) on embryonic and pregnancy outcomes following IVF and single vitrified-warmed blastocyst transfer

T. Okubo[1], K. Omi[1], T. Hayashi[1], N. Onda[1], T. Segawa[1]

[1]Shimbashi Yume Clinic, Advanced medical research institute of fertility, Tokyo, Japan

Study question: Do anti-centromere antibodies (ACAs) negatively influence embryonic and pregnancy outcomes following IVF and single embryo transfer?
Summary answer: ACA negatively influences embryonic outcomes following IVF, while pregnancy is not affected once expanded blastocysts are produced and transferred.
What is known already: ACA is a member of the anti-nuclear antibody (ANA) family. It has been considered that ACA may be the major antibody of ANA group, which has adverse effects on clinical outcomes following IVF-ET. Since the population of ANA positive (+) and ACA positive (+) infertile patients is low, studies regarding the significance of ACA, among the types of ANA, in the possible interference with embryonic and pregnancy outcomes are limited.
Study design, size, duration: A retrospective cohort study of 601 cycles (223 patients, mean age: 39.9±4.1 years), including 560 cycles (213 patients, mean age: 40.0±4.0 years) involving ANA (+)/ACA (-) and 41 cycles (10 patients, mean age: 39.0±4.3 years) involving ANA (+)/ACA (+), was conducted between May 2017 and March 2019 in a single centre. Embryonic and pregnancy outcomes following single ET were compared among the groups.
Participants/materials, setting, methods: A total of 1,419 oocytes (ANA (+)/ACA (-): 1,224 and ANA (+)/ACA (+): 195) were retrieved. Oocyte maturation status was defined by first polar body visualization or meiotic spindle confirmation. Oocytes were inseminated using IVF or ICSI. The primary outcomes were normal fertilization, multiple nucleation (3PN<), cleavage, and good-quality blastocyst formation rates (Gardner criteria: grade >3). Secondary outcomes were clinical pregnancy (gestational sac observation) and ongoing pregnancy rates (foetal heartbeat observation).
Main results and the role of chance: The ANA (+)/ACA (-) group had normal fertilization and multiple nucleation rates of 80.0% (1389/1737) and 6.8% (118/1737), respectively, while the ANA (+)/ACA (+) group had rates of 60.2% (80/133) and 26.3% (35/133), respectively. The ANA (+)/ACA (+) group showed a significantly higher multiple nucleation rate (p<0.05). Although there were no significant differences in cleavage rates among the groups (ANA (+)/ACA (-): 75.4%, 1310/1737 and ANA (+)/ACA (+): 58.6%, 78/133), the occurrence of fragmentation in cleaved embryos was significantly higher in the ANA (+)/ACA (+) group (ANA (+)/ACA (-):42.2%, 553/1310, and ANA (+)/ACA (+):57.7%, 45/78, p<0.05). The good-quality blastocyst formation rates were significantly lower in the ANA (+)/ACA (+) group than in the ANA (+)/ACA (-) group (ANA (+)/ACA (−): 32.7%, 429/1310); ANA (+)/ACA (+): 14.1%, 11/78), p<0.05). A total of 268 and 11 cycles of single vitrified-warmed blastocyst transfer were performed in the ANA (+)/ACA (-) and ANA (+)/ACA (+) groups, respectively. No significant differences were observed between the groups in clinical pregnancy and ongoing pregnancy (ANA (+)/ACA (−):53.4%, 143/268 and 44.0%, 118/268; ANA (+)/ACA (+):72.7%, 8/11 and 54.5%, 6/11).
Limitations, reasons for caution: The main limitation of the present study was its retrospective design and small sample size. The rate of ANA (+)/ACA (+)was small (0.8%, 10/1,227) in all IVF patients during the study period. Therefore, these were the only cases of ANA (+)/ACA (+) available.
Wider implications of the findings: Oocytes in ANA (+)/ACA (+) patients showed a significantly higher incidence of multiple nucleation, which was associated with a significantly lower good-quality blastocyst formation rate. However, acceptable pregnancy outcomes in those patients would be achievable if good-quality blastocysts are obtained and transferred.
Trial registration number: None.

P-176 Effects of maternal ageing on trophectoderm transcriptome dynamics and the potential impact on embryo-endometrial interactions.

P. Ntostis[1], G. Kokkali[2], D. Iles[1], J. Huntriss[1], A. Pantou[2], K. Pantos[2], H. Picton[1], D. Miller[1]

[1]LICAMM Institute, Discovery and Translational Science, Leeds, United Kingdom ; [2]Genesis Athens Clinic, Reproductive Medicine Unit, Athens, Greece

Study question: What differences are there in the human trophectoderm transcriptome among young, intermediately aged and older women with respect to the receptive endometrium?
Summary answer: The most significant gene ontologies from young/intermediate to advanced maternal age trophectoderm involved extracellular exosomes and, when receptive endometria were considered, the regulation of apoptosis.
What is known already: Chromosomal nondisjunction is the most frequent cause of recurrent miscarriages, with women over 35 years of age also experiencing lower pregnancy rates. Euploid embryo transfer reported decreased live birth rates in women over 35 years, with women over the age of 40 experiencing significantly lower implantation and pregnancy rates. Several factors involved in embryo-endometrial communication may contribute to lower success rates, with blastocyst-secreted exosomes potentially affecting endometrial receptivity and gene expression, regulating embryo implantation. To date, no study has specifically explored the trophectoderm transcriptome with respect to the maternal age and endometrial receptivity.
Study design, size, duration: Fifteen women treated for infertility in a single IVF unit agreed to participate in this study. Trophectoderm biopsies (Day 5 blastocysts) were obtained from 4 women below 30 years (young cohort), 8 women aged 30-39 years (intermediate cohort) and 3 women over 40 years old (AMA cohort). Samples were collected over a 6-month period and RNA was isolated. Our study design also included the analysis of transcriptome data from receptive endometria.
Participants/materials, setting, methods: Library construction and deep RNA sequencing included 10 trophectoderm biopsies derived from young women (24.4±2.0 years), 16 from intermediate aged women (34.3±2.6 years) and 6 from women of advanced maternal age (AMA) (42.0±1.1 year) (mean±SD). RNA expression profiles were obtained from all 32 trophectoderm biopsies (Day 5 blastocysts). Trophectoderm characterisation and ontological analysis were followed by the investigation of the complementarity between young/AMA trophectoderm and receptive/non-receptive endometrial transcriptomes. Chromosome aneuploidy analysis was also performed.

Main results and the role of chance: RNA sequencing revealed 233 significantly differentially expressed genes (DGE) (580 transcripts), between young and AMA trophectoderm transcriptomes, with FDR ranging between 5.59 x E-08 and 0.049 in the young and 7.29 x E-06 to 0.049 in the AMA cohorts. These results correspond to log fold changes of between 0.66 and 9.97 in the young and between -0.98 and 9.24 in the AMA cohort, respectively. No significantly differentially expressed genes were revealed between young and intermediate cohorts.

Cellular component analysis of the differentially expressed genes between young and AMA trophectoderm, revealed factors potentially participating in molecular trophectoderm-endometrial communication, including 42 genes strongly associated with extracellular exosomes (FDR 8.2E-06). Considered alone, the AMA cohort did not reveal any significant ontologies, however a trend for higher representation of mitotic spindle and cytosol ontologies was noted. These results may suggest the role of extracellular exosomes in the communication between trophectoderm and endometrium in human. When additional endometrial gene expression data were included in our analysis, the regulation of apoptosis was strongly represented in both embryonic and endometrial transcripts, suggesting that this could be an important process linked to successful implantation.

Limitations, reasons for caution: The relatively high variation in trophectoderm RNA expression presents a reason for caution. However, the relatively low variation within the young and AMA cohorts and the fact that a minimum of 6 samples were analysed (from at least 3 different women per cohort), minimized the potential impact on our analysis.

Wider implications of the findings: Understanding the effects of maternal ageing on the trophectoderm RNA, could identify processes that delineate normal embryo development. Considering also the receptive endometrial transcriptome, further insights into the mechanisms involved in successful implantation may be revealed. These findings have the potential to increase the low implantation rates of AMA women.

Trial registration number: N/A

P-177 Clinical application, safety and efficacy of Day 4 biopsy for preimplantation genetic testing. A retrospective cohort analysis.

G. Liperis[1], H. Swearman[1], J. Crittenden[1], C. Sjoblom[1]

[1]Institute of Reproductive Medicine- University of Sydney, Westmead Fertility Centre, Sydney, Australia

Study question: To evaluate the safety and efficacy of Day 4 embryo biopsy

Summary answer: The developmental potential of embryos and cycle outcomes suggest that day 4 biopsy is a safe and effective stage at which to perform embryo biopsy.

What is known already: Embryo biopsy routinely takes place on day 3 (cleavage stage) or day 5-6 (blastocyst stage) of embryonic development; with the latter preferred due to the ability to biopsy more cells, providing increased genetic material for analysis. A limitation of blastocyst biopsy is that several embryos capable of generating a pregnancy may not be biopsied if they fail to reach the blastocyst stage. In a recent study, transfer of euploid embryos following morula biopsy on day 6 has shown significantly lower implantation rate and birth rate compared to transfer of euploid blastocysts.

Study design, size, duration: A retrospective analysis was undertaken of patients having day 4 embryo biopsy at a single Centre between 2014 and 2017. The reasons for PGT-A included implantation failure, history of recurrent miscarriages and advanced maternal age, whilst PGT-M was employed for specific gene defects and PGT-SR for structural rearrangements. Average patient age was 36.4 ± 4.8 years. A total of 152 cycles of PGT from 148 couples were biopsied on day 4 (1890 embryos).

Participants/materials, setting, methods: Participants were consented patients undergoing IVF/ICSI cycles combined with PGT. Following fertilisation, fresh or frozen-thawed embryos, cryo-preserved at the 2PN stage for banking purposes, were cultured to day 4. On day 4, all embryos suitable for biopsy were decompacted in Ca^{2+}/Mg^{2+} free medium and 2-6 cells were removed for analysis. The genetic analysis was performed on the same day and euploid/non affected embryo(s) were either transferred on day 5 or vitrified.

Main results and the role of chance: Couples had an average of 12.4 ± 4.1 embryos biopsied with an average of 2.7 ± 1.3 cells per embryo aspirated. Following genetic analysis, 2.5 ± 1.9 euploid/normal embryos available per couple (22.6% per couple, 451/1890 total embryos). Clinical pregnancy following the day 4 biopsy was 44.5% (53/119, 1.1 embryos per ET) for con-current transfers and 36.2% (17/44, 1.1 embryos per ET) for follow-up FET cycles resulting in overall clinical pregnancy of 42.9% (70/163) and 66 live births. Neonatal outcomes included five preterm deliveries (5/63, 7.9%) with zero babies born with small for gestational age weight and zero days in NICU for deliveries ≥37 weeks of gestation (0.03 ± 0.2 days). A high proportion of biopsied embryos per patient with no genetic abnormalities reached the blastocyst stage (79.2%) when assessed on day 5. However, a large proportion of utilised embryos per patient did not reach the expanded blastocyst stage on day 5 (45.4%). A total of 11 live births resulted from day 4 biopsy method from embryos that would otherwise not have met the requirements for undergoing a day 5 blastocyst biopsy.

Limitations, reasons for caution: The results represent the experience gained from current practice and not of a prospective controlled study. No embryos were cultured to day 6, therefore the developmental potential of certain embryos classified as not expanded blastocysts at day 5 of embryo development is unknown.

Wider implications of the findings: Day 4 embryo biopsy can be a safe and effective stage for obtaining genetic material for PGT. Therefore this method can be used as an alternative approach to current embryo biopsy practices or used in combination with blastocyst biopsy when there is a small cohort of available embryos for biopsy

Trial registration number: Not applicable

P-178 Does assisted hatching of vitrified blastocysts increase clinical pregnancy rates? A randomised controlled trial

C. Cordero Rosales[1], S. Cortés[1], C. Andrés[1], R. Pandolfi[1], C. Rodriguez[1], M. Saladino[1], J. Harper[2]

[1]Clínica Tambre, FIV Labotary, Madrid, Spain ;
[2]Institute for womwn`s health, Universyty College, London, United Kingdom

Study question: Does assisted hatching (AH) of vitrified blastocysts increase clinical pregnancy rates?

Summary answer: Our study found that AH of warmed vitrified blastocysts had no effect on clinical pregnancy rates.

What is known already: The use of AH has been controversial. Some studies have shown a benefit of AH on clinical pregnancy rates for various groups of patients, including those with cryopreserved embryos, but these effects are not seen on live birth rates. Other studies have shown no difference at all. Few studies have looked at the effects of AH on vitrified blastocysts.

Study design, size, duration: This randomised controlled trial was performed between September 2018 and December 2019. 210 patients undergoing blastocyst warming were randomly allocated into a control or AH group. All patients had a single blastocyst transfer (sET). Patients undergoing PGT-A, day 3 frozen embryo transfers and double embryo transfers (dET) were excluded. T-test was performed as statistical analysis, and $p<0.05$ were considered statistically significant.

Participants/materials, setting, methods: The embryos were frozen at the blastocyst stage using the open system, Kitazato vitrification and thawing protocol. Only blastocysts of good quality were frozen (AA,AB,BA,BB) using the Gardner scoring system. Blastocysts were frozen for between 4 days and 4 years. After warming, the AH was performed immediately with the Octax-Laser at 2.6ms performing in between 2-3 pulses. Blastocysts were cultured for a minimum of 2 hours before transfer. A routine embryo transfer was performed.

Main results and the role of chance: 132 patients were allocated into the AH group and 126 to the control group. The age of the woman's egg whose blastocyst was frozen ranged from 18-45 and there was no difference between the AH and control group.

For the AH group, 70 patients had a positive hCG (53.03%), and 55 had a confirmation of clinical pregnancy by ultrasound scan by a positive heart beat (41,67%). 9 of the clinical pregnancies had a miscarriage (13%). To date, from the ongoing pregnancies there are 16 live births. For the control group, 60 patients had a positive hCG (47,61%), and 57 had a confirmation of clinical pregnancy by ultrasound scan by a positive heart beat (45,23%). 15 of the clinical pregnancies had a miscarriage (25%). To date, from the ongoing pregnancies there are 10 live births.

There were no statistical differences after comparing positive B-HCG from patients undergoing AH and those that were not (p:0.45; 95% CI). There were no significant differences in the outcomes between groups (p:0.16 95% CI).

Limitations, reasons for caution: Currently we do not have all the live birth rate for this study.
Wider implications of the findings: There has been much ongoing debate about the use of AH in clinical embryology. In our clinic, we no longer perform AH routinely after blastocyst warming. Clinics need to carefully consider if they are charging for this procedure.
Trial registration number: Not applicable

P-179 Cell-free mitochondrial DNA facilitates granulosa cells apoptosis and reduces developmental competence of oocytes from aged mice

Y. Liu[1], Q. Shen[1], W. Xiang[1], L. Zhang[1]

[1]Tongji Medical College- Huazhong University of Science and Technology, Institute of Reproductive Health and Center for Reproductive Medicine, Wuhan, China

Study question: Is the addition of cell-free mitochondrial DNA(cf-mtDNA) to the culture medium affects mouse granulosa cells function and developmental competence of oocytes matured *in vitro*?
Summary answer: Cf-mtDNA may bind to TLR9 and activate NF-κB /MAPK signal pathways, subsequently induces granulosa apoptosis and reduces developmental competence of oocytes from aged mice.
What is known already: Quantitative analysis of cf-mtDNA in human follicular fluid showed that the relative content of cf-mtDNA was positively correlated with patient age, and the relative content of cf-mtDNA may be closely related to oocyte developmental competence. MtDNA, like bacterial DNA containing a large number of unmethylated CpG sequences, can causes inflammation as a damage-associated molecular pattern molecule when it is released out of the cells.
Study design, size, duration: C57BL/6J mouse ovarian granulosa cells and cumulus-oocytes complex were cultured accompanied with cf-mtDNA *in vitro*. Mitochondrial function, expression levels of genes and proteins related apoptosis and signal transduction pathway in granulosa cells, estrogen and progesterone levels in the media were tested. 2-cell embryos and blastocysts were also counted after maturation and fertilization *in vitro* of oocytes from young and aged mice.
Participants/materials, setting, methods: Mitochondrial function was assessed by measuring mitochondrial membrane potential, ATP levels, mtDNA copy numbers, and the apoptosis genes and apoptosis pathway proteins expression were evaluated by Western-blot assay, the mRNA expression of the apoptosis genes were also tested. Chemiluminescence immunoassay (CLIA) was used to detect the levels of estrogen and progesterone.
Main results and the role of chance: Granulosa cells in the cf-mtDNA treatment group had lower ATP content ($P<0.05$),higher apoptotic cells percentage ($P<0.01$), and higher mRNA and protein levels of apoptosis-related factors than the control. Also, the expression levels of TLR9, NF-κB and MAPK proteins in granulosa cells were significantly increased in the cf-mtDNA treatment group ($P<0.05$), suggesting that cf-mtDNA induced apoptosis of granulosa cells was associated with the binding of cf-mtDNA to TLR9 receptors and then activation of NF-κB and MAPK pathway. Blastocyst formation rate of oocytes matured *in vitro* from aged mice decreased significantly ($P<0.05$), however, developmental competence of oocytes from young mice had not been affected when cf-mtDNA was supplemented into *in vitro* maturation medium, suggesting that increase of cf-mtDNA in media for aged mouse oocytes could be a factor to affect oocyte quality.
Limitations, reasons for caution: Findings *in vitro* might not be suitable for *in vivo*.
Wider implications of the findings: This study suggests that extracellular mtDNA could cause to damage to granulosa cell function and subsequently decrease of oocyte developmental competence.
Trial registration number: no

P-180 An unbiased classification system including both fresh and first freeze-all cycles for reporting and comparing IVF results

P. Saldeen[1], K. Källén[2]

[1]Institution of Clinical Sciences Malmö- Lund University, Nordic IVF Malmö, Malmö, Sweden ;
[2]Institution of Clinical Sciences Lund- Lund University, Center of Reproductive Epidemiology- Lund University, Lund, Sweden

Study question: Is it more justified to include not only fresh, but also first freeze-all cycles in a classification system for reporting IVF result
Summary answer: In a series of 31017 oocyte retrievals, the 22.7% delivery rate after fresh embryo transfers (ET) increased to 25.7% when including also first frozen ETs
What is known already: Based on 12732 fresh IVF cycles, we presented in 2010 a "totally inclusive and mutually exclusive" clinically relevant classification system for reporting IVF results, imitating Robson's 10-group model for classifying cesarean sections. The most important factors for predicting delivery after IVF were female age, number of aspirated oocytes, cumulative gonadotropin dose, number of previously failed IVF cycles, and previous IVF delivery. The system has since then been used in Sweden for open and fair comparisons of delivery rates between IVF clinics, and for revealing temporal changes. However, after introduction of the freeze-all strategy the system is no longer "totally inclusive
Study design, size, duration: The material was obtained from the Swedish national quality register for assisted reproduction (Q-IVF) during 30 months 2015-2017. The register includes six public and 12 private clinics. The delivery rate per oocyte retrieval and ET, respectively,was calculated separately for fresh ETs and when including first frozen ETs (FFET) within 6 months when freeze-all was done.
Participants/materials, setting, methods: 31017 oocyte retrievals resulted in 25987 ETs and 7967 deliveries. The revised model included 10 groups: Female age (1) >42 years; (2) 40-41; (3) 36-39, previously ≥3 failed fresh IVF cycles; (4) <36, ≥3 failed fresh IVF cycles; (5) 36-39, previous IVF child; (6) < 36, previous IVF child; (7) 38-39, no previous IVF child; (8) 36-37, no previous IVF child; (9) 31-35, no previous IVF child; (10) ≤30, no previous IVF child.
Main results and the role of chance: By adding FFETs performed within 6 months after oocyte retrieval to fresh ETs, i.e. including FFETs when cycle cancellation was done due to freeze-all, the rate of delivery per oocyte retrieval increased from in mean 22.7% (95% CI 22.3-23.2; range 7.5-35.8%) to 25.7% (95% CI 25.2-26.2; range 8.4-39.1%). The mean increase was 2.4% with the lowest increase in group 2 (0.6%) and the largest in group10 (5.0%). The corresponding figures for delivery per ET were 27.1% (95% CI 26.6-27.7; range 9.6-39.4%) and 30.7% (95% CI 30.1-31.2; range 10.8-43.1%). For both delivery per oocyte retrieval and delivery per ET, the lowest success rates were found in group 1 (Fresh ET 7.5%, FFET after cancelled cycle 8.4% per oocyte retrieval; 9.6% respectively 10.8% per ET) and the highest in group 6 (Fresh ET 35.8%, FFET 39.1% per oocyte retrieval; fresh ET 39.4%, FFET 43.1% per ET).
Limitations, reasons for caution: The classification system should include deliveries after freh ETs and FFETs, but not both in an individual, and exclude multiple ETs of frozen/thawed embryos. A Swedish consensus decided the 6 month limit, since later FFETs may happen very occasionally.
Wider implications of the findings: Our 10-group classification system will reflect variations in IVF results over time in a clinic, and between clinics on national and international levels. The system can reveal inferior results in specific groups, to be scrutinized and improved. Further sub-classifications relative to ovarian sensitivity indices are optional.
Trial registration number: Not applicable

P-181 Embryo Biopsy on Morphologically Poor Quality Blastocyst – will it help improve the Reproductive Outcomes?

S.T. Mathew[1], K. Mantravadi[2], D.G. Rao[3]

[1]Oasis Fertility, Embryology, Vishakapatanam, India ;
[2]Oasis Fertility, Embryology, Hyderabad, India ;
[3]Oasis Fertility, Medical, Hyderabad, India

Study question: Embryo biopsy on morphologically poor quality blastocyst – will it help improve the reproductive outcomes?
Summary answer: Transfer of PGT-A screened euploid, morphologically poor quality blastocyst (MPB) can improve the overall pregnancy outcome compared to non-screened MPBs.
What is known already: Traditionally embryo quality based on morphological parameters is considered as a major predictive factor for the pregnancy outcome in ART. Particularly, a strong association has been reported between the blastocyst morphology and pregnancy outcomes. However, morphological assessment is still not an ideal way to select and de-select embryos to optimize reproductive outcomes. Currently, the focus is to find a method, which enables us to pick the best embryo for optimal results.
Study design, size, duration: All autologous frozen blastocyst embryo transfers (FET) with known pregnancy outcomes from January 2016 to March 2019 were analyzed. Patients who had a day 5/6 transfer with one or two MPBs were

considered in this study. A total of 86 patients underwent FET with non PGT-A MPB and 18 patients with PGT-A MPB. Statistical analysis of the obtained data was performed to find an association between the PGT-A of MPB and reproductive outcomes.

Participants/materials, setting, methods: A total of 104 patients were recruited for the retrospective study. Group I had 86 patients who underwent FET with non PGT-A MPB and group II had 18 patients with PGT-A MPB.

Among the clinical outcomes clinical pregnancy rate (CPR), miscarriage rate (MR), implantation rate (IR) and live birth rate (LBR) were included and perinatal outcomes includes average birth weight (BW), preterm delivery and delivery complications.

Main results and the role of chance: The CPR, MR, IR and LBR between group I and group II were found to be 43.02% vs. 66.67%, 15.12% vs. 16.67%, 26.53% vs. 66.67% and 16.46% vs. 50% respectively. Among the perinatal outcomes there was no incidence of low birth weight (LBW) or any congenital anomalies. Average birth weights and preterm delivery rates were 2.8 vs. 2.5kgs and 1.27% vs. 0% respectively. Clinical outcomes seem to be better with PGT-A group. Perinatal outcomes were comparable between both the groups. Results obtained in this study re-assure that embryo biopsy technique on poor morphology embryos doesn't seem to affect the perinatal and neo-natal outcomes. PGT-A on MPB can be used as an additional tool to pick the best embryo.

Limitations, reasons for caution: Retrospective study, Small sample size, Relative subjectivity of embryo scoring.

Wider implications of the findings: Our findings may help to reassure women that morphologically poor quality embryos can be screened for PGT-A and if euploid, can offer the best reproductive outcomes. Apart from morphology, PGT-A can be an additional tool for selecting and de-selecting the embryos for optimizing pregnancy outcomes.

Trial registration number: not applicable

P-182 Acute exposure to Etoposide does not activate DNA damage checkpoints in Meiosis I of human oocytes.

M. Botzaki[1], K. Agapitou[1], E. Nikitos[1], S. Kallergi[1], K. Niaka[2], P. Marangos[2]

[1]INSTITUTE OF LIFE-IASO Maternity hospital, IVFunit, Athens, Greece ; [2]University of Ioannina, Department of Applications and Technology-, Ioannina, Greece

Study question: Can spindle disruptions induced by Etoposide, a topoisomerase II inhibitor, activate DNA damage checkpoints in Meiosis I of human oocytes?

Summary answer: DNA damage accumulated in meiosis-I, does not prevent polar body extrusion and therefore could persist in morphologically normal metaphase-II oocytes.

What is known already: Severe spindle disruption activated the checkpoint to prevent polar body extrusion, moderate spindle insults fail to prevent polar body extrusion, causing defective chromosome segregation that likely leads to uneuploidy. Studies showed that mouse oocytes with damaged DNA can resume meiosis and undergo Germinal Vesicle Breakdown (GVBD), but then arrest in metaphase of meiosis-I in a process involving Spindle Assembly Checkpoint (SAC) signalling.Such a mechanism could help prevent the generation of metaphase-II (Met-II) eggs with damaged DNA.Little has been known about the spindle checkpoint in human oocytes.

Study design, size, duration: Donor immature oocytes aged <35years in a time frame of two years.

Experiments performed: 8 experiments involving 4 control and 8 oocytes treated with 100μg/ml Etoposide for 1h, from 8 individual donors.

26 experiments involving 54 control and 57 oocytes treated with 20μg/ml Etoposide for 1h, from 16 individual donors.

Histone H2AX and is used as an immunofluorescence marker for the detection of double-strand breaks. Draq7 is used for DNA staining. GV-stage oocytes are fixed immediately after treatment.

Participants/materials, setting, methods: Human oocytes were recovered from donor ovaries. Germinal Vesicle (GV)-stage oocytes were treated with high (100μg/ml) and lower (20μg/ml) concentrations of Etoposide for 1h. Following Etoposide treatment, treated and control oocytes were placed in the incubator at 37.5oC under 7.1% CO2.

Live imaging by time-lapse monitoring began immediately after oocyte transfer to the incubator, by the use of the Primo Vision. All data analysis was performed using ImageJ/Fiji.

Main results and the role of chance:

- Lower concentrations of Etoposide cause DNA damage but most oocytes progress through meiosis-I and subsequently extrude the first polar body (PB1) to form a metaphase-II egg, revealing the absence of a DNA-damage-induced SAC response.
- DNA damage accumulated in meiosis-I, such as could occur during in vitro maturation procedures, does not prevent polar body extrusion and therefore could persist in morphologically normal metaphase-II oocytes.

Limitations, reasons for caution: no limitations or reasons for caution

Wider implications of the findings: Multiple aspects of the clinical IVF environment could potentially damage the DNA of cultured oocytes.

This may explain poor embryo quality and repetitive implantation failures in many of the cases.

Trial registration number: not applicable

P-183 Oxygen concentration in culture media equilibration: does it have an impact on pregnancy rate?

E. De Martino[1], P. Filardi[1], G. Terrado[2], N. Passi[2], M. Gomez Peña[1], M. Papayannis[1], J. Maidana[1], I. De Zúñiga[2], L. Kopcow[2], M. Horton[2], A. Oubiña[2], F. Sobral[2], C. Bisioli[1]

[1]Pregna Reproductive Medicine, Embryology Laboratory, Buenos Aires, Argentina ; [2]Pregna Reproductive Medicine, Reproductive Medicine, Buenos Aires, Argentina

Study question: Can we culture human embryos at low oxygen in media that has been equilibrated overnight at atmospheric oxygen without affecting pregnancy rates?

Summary answer: Pregnancy rates are not affected when embryos are cultured at low oxygen in media that has been equilibrated at atmospheric oxygen concentration.

What is known already: Low oxygen has been shown to be crucial to culture embryos up to the blastocyst stage. Despite this, many laboratories still culture embryos in atmospheric conditions. Others use the old incubators for media equilibration overnight in order to save space in the low oxygen ones. When oocytes or embryos are placed into these high oxygen dishes they will be exposed to a temporary atmospheric state while low concentration is finally reached. Although it is generally pointed out that embryo culture must be entirely done at low oxygen, there are few studies proving that this is true.

Study design, size, duration: This prospective cohort study included 146 women performing fresh ART treatment between May 2018 and July 2019. Exclusion criteria included couples with severe male factor and donor cycles.

Participants/materials, setting, methods: According to the culture media equilibration methods used in our laboratory (overnight dishes at atmospheric or low oxygen) and the incubators space availability, patients were randomly divided into two groups:

- Group 1: Atmospheric oxygen (n=94)
- Group 2: Low oxygen (n=52)

Primary outcome was clinical pregnancy rate. Secondary outcome was implantation rate. No differences in patient age, insemination method and infertility diagnosis were observed. Chi-square and Student's t test were used as appropriate.

Main results and the role of chance: No significant differences were found in clinical pregnancy rate (23.4% vs. 21.1%) and implantation rate (15.4% vs 14.5%) between groups. Treatment outcome seems not to be affected by an exposition of around 4 hours to an atmospheric oxygen concentration in the beginning of the culture. Although several studies suggest a negative impact of oxidative stress in embryo development, there are still few regarding the effect of a brief exposition to atmospheric oxygen. Nevertheless it is generally recommended by personal communications that embryo culture must be done at low oxygen since the very beginning.

Limitations, reasons for caution: Our study is limited by its sample size and the need to go beyond pregnancy and implantation rates. In this sense it would be important to also study the possible effect that these two different

oxygen concentrations may have on the embryo physiology or on the newborn health.
Wider implications of the findings: To the best of our knowledge, this is the first analysis comparing two media equilibration conditions using atmospheric oxygen incubators as a possible viable option in cases of new technologies access limitations. More studies are necessary to elucidate the possible effects of transient oxygen concentration variation on human embryo development.
Trial registration number: Not applicable

P-184 The usefulness of metaphase I oocytes for intracytoplasmic sperm injection

J.P. Bilibio[1], A.J.C. Meireles[2], Y. Maciel[3], P. Sales[2], F. Nascimento[2], P.L. Lorenzzoni-[4]

[1]Universidade Federal do Pará, Ginecologia e obstetrícia, Belém ;
[2]Clínica de Reprodução Assistida Pronatus-, Reprodução Assistida, Belém, Brazil ;
[3]Universidade Federal do Pará, Ginecologia e obstetrícia, Belém, Brazil ;
[4]Grupo de Pesquisa Bilibio, Reprodução Assistida, Belém, Brazil

Study question: The aim was to evaluate the fertilization rate and blastocyst formation rate of oocytes in metaphase I (MI) in women who underwent controlled ovarian hyperstimulation for intracytoplasmic injection.
Summary answer: MI oocytes collected at stage MI after controlled ovarian hyperstimulation that did not mature in vitro to MII have a blastocyst formation rate of 0.6%.
What is known already: It is known that an increased pregnancy rate is directly related to the number of embryos in the blastocyst stage obtained by the in vitro fertilization (IVF) cycle, and this number depends on the quantity and quality of MII oocytes collected. As the number of these oocytes is inversely proportional to the age of the patient, the use of MI oocytes for IVF, especially in couples with advanced age, has been discussed as an alternative.
Study design, size, duration: A prospective cohort study was performed among women from whom at least 1 MI and 1 MII oocyte was obtained after controlled ovarian hyperstimulation for intracytoplasmic sperm injection during the study period,June 2018 to June 2019.

We collected 1907 oocytes from 164 women (1291 MII, 352 MI and 258 prophase I or atresic)
Participants/materials, setting, methods: After oocyte classification, the MII and MI oocytes were incubated for 4 hours.

After 4 hours in the single step balanced embryonic culture medium (Global Total), 1649 oocytes were fertilized by intracytoplasmic injection (ICSI), of which 1291 were in the MII stage (Group MII); of the 358 oocytes in the MI stage, 205 matured to MII oocytes (Group MI-MII), and 153 remained in stage MI (Group MI).
Main results and the role of chance: After 4 hours, the maturation rate was 57.2%; 205 matured in vitro to MII (Group MI-MII) and 153 remained in stage MI (Group MI).

The normal fertilization rate was directly associated with oocyte maturation, with rates of 79.1%, 60.2%, and 31.9% in MII, MI-MII and MI oocytes, respectively (P <0.001).

The MI group had an odds ratio (OR) of 7.6 (IC 5.2 - 11.2, P <0.001) for abnormal fertilization compared with the MII group.

The risk of embryo polyploidy was higher in the MI group, with an OR of 2.6 (1.28-5.41,P = 0.008), than in the MII group.

The blastocyst formation rate was directly associated with oocyte maturation: 36.4% for MII, 11.4% for MI-MII and 0.6% for MI.

MI oocytes had an OR of 86.9 (CI 12.1 - 623.4, P <0.001) for not forming a blastocyst compared with MII oocytes.
Limitations, reasons for caution: We found a decrease in the blastocyst formation rate from MII oocytes but no difference in MI-MII oocyte in patients over 40 years of age. Maternal age does not seem to influence the rate of blastocyst formation from MI-MII oocytes, but these results need to be confirmed in other studies.
Wider implications of the findings: Oocytes collected at stage MI after controlled ovarian hyperstimulatio that do not mature to MII in vitro have a blastocyst formation rate of only 0.6%, so their use should be discouraged. On the other hand, the use of MI-MII oocytes may increase the amount of blastocysts available for embryo transfer.
Trial registration number: 55625115.0.0000.0018

P-185 The non invasive embryoscope morphokinetic kidscore grading system and PGT-A ploidy status

P. Sopaboon[1], M. Tuntipoj[1], P. Suanbooncharoen[1], S. Chanchamroen[1], W. Quangkananurug[2], S. Seweewanlop[1], P. Assawakittananon[1], T. Thongsook[1]

[1]Safe fertility Center, Laboratory, Bangkok, Thailand ;
[2]Safe fertility Center, Medical Director, Bangkok, Thailand

Study question: Could the KIDscore number indicate the risk of aneuploid? Does the euploid and aneuploid embryos differ in KIDscore number?
Summary answer: There is no particular KIDscore number in indicating euploid embryo. The euploid and complex aneuploid embryos were statistical significant difference in KIDscore number.
What is known already: It has been shown that euploid embryo tend to implant. The invasive biopsy followed by PGT-A is widely use to identify the ploidy status of embryo. The non-invasive embryo selection is expected to predict ploidy status to enhance the possibility of implantation. The time-lapse incubator recently play a role in culturing and selecting the best embryo to transfer. EmbryoScope(Vitrolife) has launched the KIDscore system to be the evaluation tool for scoring the embryos according to their statistical viability. The time-lapse incubator combined with the software is costly and not much data have been published about the use of this algorithm.
Study design, size, duration: Randomized prospective study. 205 blastocysts in IVF/PGT-A cycle from couples attending an independent IVF clinic (Safe Fertility Center, Bangkok, Thailand) between April to May 2019 were enrolled in this study.
Participants/materials, setting, methods: All embryos were cultured in time-lapse incubators (EmbryoScope, Vitrolife). Embryo grading was done by both morphology grading (ACE/NEQAS system) and morphokinetic grading (KIDscore, Vitrolife). The KIDscore morphokinetic parameters observed were PN assessment, PN fading, time (t) to 2, 3, 4, 5 and 8 cells, tSB and tB followed by morphology grading of ICM and TE. The blastocysts were biopsied on Day 5/6 and analysed by PGT-A(NGS, Illumina).
Main results and the role of chance: The blastocyst formation rate, blastocyst analyzed for PGT-A rate were 64.50% and 86.13% respectively. Of the 205 blastocysts, 134 were euploid, 14 were mosaic, 33 were single chromosome aneuploid, 19 were complex chromosome aneuploid and 5 were DNA degradation. The overall KIDscore average range was 6.0±2.25. The euploid embryos had 6.27±2.24 on average in KIDscore number with 95% Confident Interval (CI):5.89-6.66. While the mosaic, single chromosome aneuploid, complex chromosome aneuploid and DNA degradation were 6.04±2.29 (95% CI:4.72-7.37), 5.75±2.17 (95% CI:4.98-6.52), 4.99±2.34 (95% CI:3.86-6.12) and 5.42±2.01 (95% CI:2.92-7.92).There are statistically significant difference in euploid and complex chromosome aneuploid group (P=0.021)in KIDscore number. The KIDscore number 8.0-9.9 bound to be euploid, mosaic, single chromosome aneuploid and complex chromosome aneuploid as 76.74%, 9.30%,13.95% and 9.30% respectively. Whereas the mid-grade KIDscore number 4.0-6.0 had 64.58%,10.41%, 27.08% and 14.58% in euploid, mosaic, single chromosome aneuploid and complex chromosome aneuploid respectively. The data could imply that the high grade KIDscore number has more chance to be euploid than the mid-grade and low-grade score.
Limitations, reasons for caution: The current sample size is small and data were collected from a single clinic which has a limited generalizability and potential bias. As routinely use is running, we will report on a larger sample size to provide more reliable effectiveness.
Wider implications of the findings: When selecting the embryo to transfer, considering not only the morphology scoring, the other scoring may benefit to help select the best embryo to get the best chance to implant. The non invasive evaluation tools could be consider to use as it less harming to the embryo.
Trial registration number: not applicable

P-186 Effect of different equilibration times on clinical and neonatal outcomes in human blastocyst vitrification

S. Mitsuhata[1], Y. Fujii[1], Y. Endou[1], M. Hayashi[1], H. Motoyama[1]

[1]Kurashiki Medical Clinic, IVF center, Kurashiki, Japan

Study question: What are the effects of shortened or prolonged equilibration time during human blastocyst vitrification?

Summary answer: A shorter (5–11 min) equilibrium time is sufficient for vitrifying smaller, non-expanded blastocysts.
What is known already: In human embryo vitrification, the duration of embryo's suspension in the vitrification solution is a critical consideration. This process should be strictly controlled within 1 min. However, exposure to the equilibration solution is flexible. A prolonged exposure to the equilibration solution may be harmful and may influence the embryonic developmental potential, whereas a shortened exposure may impair the penetration of cryoprotectants into the blastomeres. With regard to human blastocysts, few studies examine the effects of different equilibration times on clinical and neonatal outcomes.
Study design, size, duration: This was a retrospective study based on data collected between November 2008 and November 2015. A total of 225 blastocysts, 105 non-expanded blastocysts and 120 expanded blastocysts, obtained from 173 patients were analyzed. Non-expanded and expanded blastocysts were divided into 2 groups according to the equilibration time: 5–11 min and 12–15 min. Clinical and neonatal outcomes of warmed blastocysts were compared in each group.
Participants/materials, setting, methods: The blastocysts were vitrified and warmed following the Cryotop method. Blastocysts with blastocoels occupying less than half of the embryo volume and showing no signs of zonae thinning or expansion were categorized as non-expanded blastocysts. Blastocysts with thinning zonae and blastocoels that occupied greater than half of the embryo volume were identified as expanded blastocysts. Hatching blastocysts were classified as expanded blastocysts.
Main results and the role of chance: Both equilibration times (5–11 min and 12–15 min) in the non-expanded blastocyst group resulted in effective vitrification. There were no differences in the survival (96.9% vs. 92.5%, 95% CI: 0.48–13.4), implantation (20.0% vs. 20.0%, 95% CI: 0.38–2.62) and live birth rates (12.3% vs. 17.5%, 95% CI: 0.23–1.92) between the groups. While the results for the expanded blastocysts, the survival (86.7% vs. 96.7%, 95% CI: 0.05–0.99, P<0.05), implantation (23.3% vs. 45.0%, 95% CI: 0.17–0.81, P<0.05) and live birth rates (16.7% vs. 38.3%, 95% CI: 0.14–0.75, P<0.01) were significantly decreased in the 5-11 min group compared with 12-15 min group. There were no differences in gestational age, birthweight, proportion of male babies, rates of cesarean section, and congenital abnormalities.
Limitations, reasons for caution: This study is limited by sample size. There is possible dispersion of the embryo quality and cause of infertility. Further prospective randomized studies will be needed to confirm the findings of this study.
Wider implications of the findings: Prolonged exposure to cryoprotectants may incur harmful effects on embryonic development; thus, shortening exposures to equilibration solution are desirable. This study shows that outcomes are satisfactory if unexpanded blastocysts are vitrified with shorter exposure times. Therefore, reducing the cytotoxicity of cryoprotectants and vitrification process is completed faster.
Trial registration number: not applicable

P-187 Cleavage stage versus blastocyst stage embryo transfer in Bologna poor responders: a retrospective analysis.

J. Errazuriz[1,2], F. Pais[1], A. Racca[2], G. Verheyen[2], I. Filselcker[1], P. Gonzalez[1], H. Tournaye[2], C. Blockeel[2]

[1]Centre for Reproductive Medicine, Departamento de Ginecología y Obstetricia- Clínica Alemana- Facultad de Medicina- Universidad del Desarrollo., Santiago, Chile ;
[2]Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel- Vrije Universiteit Brussel., Brussels, Belgium

Study question: Do poor ovarian responders (POR) with only one available embryo on day 3, achieve higher pregnancy rates by transferring cleavage or blastocyst stage embryos?
Summary answer: In POR with only one embryo available, transferring the embryo on day 5 was found to achieve higher live birth rates compared with day 3.
What is known already: IVF/ICSI cycles in POR remain challenging regarding ovarian stimulation, and are characterized by low live birth rates (LBR) and high cancellation rates. Traditionally, embryo transfer was carried out on day 3; however, in recent years there has been a shift in practice towards embryo transfer at the blastocyst stage. Nevertheless, these studies have not addressed the dilemma of how to proceed when only one embryo is available, as frequently observed in POR. Therefore, the implantation rate and pregnancy outcomes in relation to an individual embryo remain unclear.
Study design, size, duration: This is a retrospective cohort study including 622 patients with POR who underwent IVF/ICSI cycles in two reproductive centers (from Belgium and Chile) between January 1st 2011 and December 1st 2018.
Participants/materials, setting, methods: All women who fulfilled the Bologna criteria POR and underwent IVF/ICSI cycles with only one available embryo on day 3, were included. The decision to transfer one embryo on day 3 or day 5 was made by the treating clinician and only Grade I-III were transferred. Embryo quality was similar in both groups. We excluded patients who did not have an available embryo on day 5.
Main results and the role of chance: 622 Bologna criteria POR patients with only one available embryo on day 3 were included in the analysis: 498 were categorized to the "day 3 group" and 124 to the "day 5 group". There were no baseline differences between the groups. In the day 5 group, higher clinical pregnancy rates per started cycle (28% versus 19%, P <0.001) and higher live birth rates (18% versus 11%, P = 0.043) were obtained compared to the day 3 group. Multivariate logistic regression analysis for relevant confounders, showed that the only independent variable that significantly associated to higher LBR was the day of the transfer (OR 1.97, 95% CI 1.12-3.44, P = 0.02).
Limitations, reasons for caution: The main limitation in our study is the retrospective design. In addition, the decision to transfer the embryo on day 3 or day 5 was made by the treating clinician during their IVF cycle and according to the progress in embryo development, which may be a potential source of bias.
Wider implications of the findings: In POR with only one viable embryo on day 3 of embryonic development, performing the transfer on day 5 was found to achieve higher pregnancy and LBR compared with day 3 transfer. This challenges current practices to be modified to improve fertility outcomes in this specific group of patients.
Trial registration number: not applicable

P-188 Sorbitol accumulation decreases oocyte quality of aging mice through increasing oxidative stress

Y. Zhang[1], H. Liu[1], L. Li[1], Z. Yan[1], Y. Mao[1], L. Gao[1], J. Liu[1], Y. Cui[1]

[1]State Key Laboratory of Reproductive Medicine- Clinical Center of Reproductive Medicine, First Affiliated Hospital- Nanjing Medical University, Nanjing, China

Study question: Whether could the sorbitol accumulation in oocytes decrease the quality of aging oocytes ?
Summary answer: The excessive sorbitol decreases the oocyte quality of aging mice, and the sorbinil supplementation, inhibiting theproduction of sorbitol, can improve the quality of aging oocytes.
What is known already: Sorbitol is a by-product of glucose metabolism through the polyol pathway. Many studies have demonstrated that the excessive sorbitol can decrease the cellular activation due to the increased ROS.
Study design, size, duration: This is an in vitro study with COCs obtained from 9 month-old female mice, to explore the sorbitol accumulation related with oocyte quality.
Participants/materials, setting, methods: The COCs obtained from 9 month-old female mice were *in vitro* cultured with or without sorbinil, a kind of aldose reductase inhibitor as the control and the inhibitor group. In the young group, the COCs obtained from 6-8 week-old female mice were cultured the same as the control. MII oocytes were collected. The ROS and GSH were tested. The level of Aldose reductase, SOD_1 were measured. The contents of sorbitol were measured by HPLC/MS.
Main results and the role of chance: The maturity rate of oocytes in the inhibitor group was significantly higher than that in the control group (increased 16%, P<0.05). The ROS florescence intensity in the inhibitor group was decreased by 42% comparing with the control group (p<0.0001). The GSH florescence intensity in the inhibitor group was increased by 17% comparing with the control group (P<0.05). Interestingly, SOD_1 was upregulated in the inhibitor group (P<0.05). The content of sorbitol in the control group was drastically increased comparing with the other groups (P<0.001). The level of Aldose reductase was increased in the *in vitro* aging oocytes of 9 month-old female mice and the in vitro maturation oocytes of 6-8 week-old female mice (both P<0.05).

The sorbitol was accumulated in the *in vitro* aging oocytes comparing with the MII oocytes matured *in vivo* of 9 month-old female mice (P<0.05).
Limitations, reasons for caution: Sorbitol accumulation decreases the oocyte quality of aging mice, while whether the content of sorbitol in human oocytes increases during in vitro maturation is sparsely understood.
Wider implications of the findings: The excessive sorbitol may decreases the oocyte quality of aging mice, and the inhibition of sorbitol production during IVM may improve the oocyte quality by decreasing the oxidative stress.
Trial registration number: not applicable

P-189 Slowly developing blastocysts: fresh embryo transfer on day 5, on day 6 or frozen embryo transfer?

A. Reignier[1], A. Inquel[1], S. Loubersac[1], J. Lammers[1], P. Barriere[1], T. Lefebvre[1], T. Freour[1]

[1]*CHU Nantes, Biologie et médecine de la reproduction, Nantes, France*

Study question: Should slowly developing blastocysts be transferred on day 5, when expanded on day 6, or be frozen in order to optimize live birth rate (LBR)?
Summary answer: Fresh day 5 early cavitating blastocyst transfer resulted in higher LBR than fresh expanded day 6 blastocyst transfer or frozen-thawed day 6 embryo transfer.
What is known already: LBR are higher after fully expanded blastocyst fresh transfer on day 5 than after slow-growing expanded blastocysts fresh transfer on day 6. Embryo-endometrial asynchrony could explain this decrease. In this respect, some studies suggested day 6 blastocysts should systematically be frozen, but this still remains unclear. Moreover, there is no consensus about the best strategy to adopt with slowly developing blastocysts which are stil at the early cavitating stage (B1 or B2 according to Gardner & Schoolcraft criteria) on day 5, as to whether they should be transferred on day 5, 6 or vitrified on day 6.
Study design, size, duration: This was a retrospective monocentric cohort follow-up study including 306 cycles performed between 2016 and 2017 with at least 1 early cavitating blastocyst available on day 5 which was either transferred fresh on day 5 or cultured up to day 6 and transferred fresh or vitrified.
Participants/materials, setting, methods: All patients with early cavitating blastocyst on day 5 (Gardner stage B1 or B2) were included and allocated to 3 groups. Group 1 had fresh unexpanded blastocyst transfer on day 5 (n=68), group 2 had fresh full expanded blastocyst transfer on day 6 (n=57) and group 3 had frozen embryo transfer after vitrification on day 6 (n=181). LBR was the main outcome and was compared between the 3 groups.
Main results and the role of chance: Female age (33.8, 32.9, 34.1 years old) and smoking status (19.1, 14.0, 13.3%) were comparable between the groups 1, 2 and 3 respectively. Female BMI was significantly higher in group 1 than in groups 2 and 3 (26.1 versus 24.7 and 23.7 kg/m² respectively). Serum AMH level was significantly higher in group 3 than in groups 1 and 2 (3.1 versus 2.3 and 2.4 ng/mL respectively). LBR was significantly higher in group 1 (fresh transfer on day 5) than in group 3 (frozen day 6 transfer): (26.4%) versus (13.8%); p < 0.05. LBR was 14.0% for group 2 (fresh transfer on day 6) without significant difference with groups 1 and 3.
Limitations, reasons for caution: The relatively limited numbers of cycles included as well as the retrospective design of the study are significant limitations. Vitrification of early blastocysts on day 5 has not been evaluated in this study.
Wider implications of the findings: Fresh day 5 transfer should be promoted for early cavitating blastocyst rather than fresh day 6 transfer or vitrification. These preliminary results should be integrated when the embryo culture and transfer strategy is considered by the clinicobiological IVF staff, and should be used to improve patients' counselling.
Trial registration number: none

P-190 Should we transfer poor quality embryos?

A. Kirillova[1]

[1]*Federal State Budget Institution "National Medical Research Center for Obstetrics-Gynecology and Perinatology named after Academician V.I. Kulakov of the Ministry of Healthcare of Russian Federation", Department of preservation and restoration of the,*

Study question: Should poor quality embryos be transferred?
Summary answer: It is safe to transfer poor quality embryos when they are the only option for fresh ET.
What is known already: Implantation potential of poor morphology embryos is much lower than of those of good and fair quality. At the same time, there are indications that it might be safe to transfer embryos of poor quality as such transfers do not increase the rate of chromosomal abnormalities, congenital malformations of children born, or perinatal complications and mortality. However, the data available on the outcomes of IVF cycles in which poor quality embryos are transferred is limited. It is also uncertain which developmental stage: cleavage or blastocyst is more favorable for poor quality embryos to transfer.
Study design, size, duration: A retrospective analysis using individual patient data with positive controls. All patients undergoing fresh embryo transfers of poor quality embryos between 2012 and 2016. 738 poor quality embryos from 488 patients were assessed. The control group consisted of 9893 fair and good quality embryos from 5994 patients.
Participants/materials, setting, methods: IVF cycles with or without ICSI involving fresh embryo transfers of poor quality embryos on day 3 or on day 5. The main outcomes of day3 and day 5 poor quality embryo transfers were compared. We also compared the main outcomes of cycles with poor quality embryo transfers with good and fair quality embryo transfers.
Main results and the role of chance: No significant differences in the biochemical pregnancy rate, implantation rate, clinical pregnancy rate, miscarriage rate and live birth rate were found between the groups of poor quality embryos transferred on day 3 or on day 5. Although the biochemical pregnancy rate and implantation rate were lower for the group of poor quality embryos than for the control (13.9% vs 37.2%, p<0.001 and 6.9% vs 29.4%, p<0.001 respectively), statistically significant differences between the portion of the biochemical pregnancies which resulted in clinical pregnancies and the portion of clinical pregnancies which resulted in live births in both groups were not observed (72% vs 78.3%, p=0.2313 and 79.6% vs 79.3%, p= 0.9636 respectively).
Limitations, reasons for caution: This was a single-center retrospective study. Embryo morphology grading was assessed by 5 experienced embryologists, but it still remains a subjective type of evaluation.
Wider implications of the findings: Unlike the common belief, our data suggest that transfers of poor quality embryos do not increase the rate of miscarriages or stillbirths and should be considered safe. Transfer of embryos of poor morphology results in lower but still acceptable live birth rates.
Trial registration number: not applicable

P-191 Pre-maturation culture with CNP before IVM does not improve oocyte competence and embryo development in women with PCOS

Y. Lin[1], X. Zheng[1], J. Yan[1], X. Song[1], R. Li[1], J. Qiao[1], C. Ma[1]

[1]*Peking University Third Hospital, Center for Reproductive Medicine, Beijing, China*

Study question: Whether a pre-culture with C-type natriuretic peptide (CNP) before in vitro maturation (IVM) improves oocyte competence and embryo developmental potential or not?
Summary answer: Pre-maturation culture (PMC) with CNP before IVM does not improve oocyte competence and embryo development in women with polycystic ovarian syndrome (PCOS).
What is known already: Numerous studies in animal models have indicated the benefit of a pre-culture with CNP on IVM of immature oocytes. In 2017, Sánchez et al reported that a new IVM culture system enhances human oocyte competence and embryo yield and thought that the improvements were mainly attributed to the PMC step in presence of CNP. However the positive effects of CNP on IVM has not yet been repeated by other research teams.
Study design, size, duration: A prospective self-control study involved 192 cumulus–oocyte complexes (COCs) from 15 women with PCOS to evaluate effects of PMC with CNP on oocyte competence and subsequent embryo developmental potential. This study was conducted from March 2019 to November 2019.
Participants/materials, setting, methods: PCOS women undergoing laparoscopic salpingectomy for hydrosalpinx in a university-affiliated reproductive center were recruited and COCs were retrieved while surgery. COCs obtained were randomly allocated to control group (conventional IVM for 30h) or PMC group (PMC with CNP for 24h + conventional IVM for 30h). Oocyte maturation

and embryo development after ICSI were compared between groups. Cumulus cells (CCs) were collected for assessments of the expressions of *NPR2* and *GJA1* mRNA and cGMP level.
Main results and the role of chance: Fifteen patients were included in the study. Total 192 COCs were divided randomly into PMC group (n=93) and control group (n=99). Oocyte maturation rates were similar between PMC group (53.8%) and control group (47.5%, p=0.384). Subsequent fertilization rate (54.0% vs 63.8%) and available embryo rate (40.0% vs 53.2%) per mature oocyte also showed no significant difference (p=0.326 and 0.193, respectively) between groups. Six blastocysts were acquired in control group (12.8%) while no blastocyst formation in PMC group (0%, p=0.029). Expressions of *NPR2* and *GJA1* mRNA of CCs denuded from COCs were similar between two groups. No significant difference was found in cGMP level of CCs between groups too.
Limitations, reasons for caution: The sample size was not large as previous studies. As no blastocyst was acquired in PMC group, the planned analyses of morphology, cell number, aneuploidy rate, DNA methylation and imprinted genes of blastocyst were not undergone. Because of the discouraging results, imaging of cumulus-oocyte transzonal connection was not taken.
Wider implications of the findings: The results of this study found that pre-cultured with CNP before IVM did not improve oocyte competence and embryo developmental potential. Further studies exploring a better IVM culture system are still needed.
Trial registration number: 2018SZ-032

P-192 What are the best decision criteria for rescue intracytoplasmic sperm injection (ICSI), reducing the risk of artificial polyspermy?

T. Kishida[1], H. Kikuchi[1], K. Yamada[1], H. Kanno[1], R. Kishida[1], T. Sato[1], M. Yuki[1], A. Kitsu[1], S. Fumiya[1], M. Katagiri[1], M. Shinagawa[1], Y. Hiroaki[1]

[1]Sendai ART Clinic, ART Lab., miyagi, Japan

Study question: To establish the decision criteria (DC) for rescue ICSI, reducing the risk of artificial polyspermy using time-lapse imaging systems.
Summary answer: Postponing the observation time of the second polar body (PB) extrusion is most effective for reducing the risk of artificial polyspermy after rescue ICSI.
What is known already: The decision to perform rescue ICSI is determined by combining the observation time of the second PB extrusion following the conventional IVF and the second PB extrusion rate at that time. Artificial polyspermy, created by performing rescue ICSI to normally fertilized oocytes after conventional IVF, must be avoided. However, the optimal DC for rescue ICSI for reducing the risk of artificial polyspermy has not been established.
Study design, size, duration: This was a retrospective study conducted at Sendai ART clinic in Japan from 2017 to 2019. A total of 1480 mature oocytes, taken from 437 cycles, were cultured using a time-lapse incubator after short insemination (4 hours). These oocytes were checked from the time of insemination to the second PB extrusion and we determined the number of pronuclei.
Participants/materials, setting, methods: The target oocytes for rescue ICSI were extracted on the basis of various DC, using time-lapse images. DC were set by combining the extrusion time of second PB (<4, 4, 5, 6, or 7 hours after IVF) and the ratio of the oocytes extruding the second PB per mature oocytes (0%, 0-30%, or 0-99%). The normally fertilized oocytes rate following IVF among the extracted target oocytes were examined as the risk rate of artificial polyspermy.
Main results and the role of chance: The risk rates of artificial polyspermy, when setting the extrusion time of the second PB at <4 hours, were significantly higher than those at 4, 5, 6, and 7 hours (<4 hours: 37.0-40.8%, 4 hours: 15.0-25.8%, 5 hours: 6.3-9.0%, 6 hours: 2.0-4.3%, and 7 hours: 0-1.0%, P<0.05). The risk rates tended to decrease gradually as the extrusion time was delayed. Conversely, there was no significant difference in risk rates between the ratios of the oocytes extruding the second PB. The risk rates decreased to 0% when the extrusion time was set at 7 hours, and the ratios of the oocytes that extruded second PB were 0% or 0-30%.
Limitations, reasons for caution: This study is a single-center retrospective study. We expect the extrusion time of the second PB to change depending on the ART protocol (e.g., controlled ovarian stimulation protocol and start time of insemination).
Wider implications of the findings: This study found that rescue ICSI must be performed after 8 hours to eliminate the risk of artificial polyspermy. However, delaying fertilization may reduce the quality of the oocytes. Therefore, further studies are needed to determine new criteria for performing rescue ICSI as soon as possible.
Trial registration number: not applicable

P-193 Effect of Time Elapsed Between Thaw and Embryo Transfer on pregnancies rates in Patients with Cryopreserved-thawed embryo transfer: A Systematic Review and Meta-Analysis

L.H. Sordia-Hernandez[1], F.A. Morales Martinez[2], E. Gutierrez Orozco[1], O.H. Valdes Martínez[1], A. Flores Rodriguez[3], P. Leyva Camacho[4], N. Alvarez Villalobos[3], J.A. Zuñiga Hernandez[1]

[1]Universidad Autonoma de Nuevo Leon, Centro Universitario de Medicina Reproductiva, Monterrey, Mexico ;
[2]Universidad Autonoma de Nuevo León, Centro Universitario de Medicina Reproductiva, Monterrey, Mexico ;
[3]Universidad Autonoma de Nuevo Leon, Plataforma INVEST Medicina UANL – KER Unit Mayo Clinic KER Unit México-, Monterrey, Mexico ;
[4]Universidad Autonoma de Nuevo Leon, Plataforma INVEST Medicina UANL – KER Unit Mayo Clinic KER Unit México, Monterrey, Mexico

Study question: Does short or long culture time between thawing and transfer of cryopreserved embryos have an impact on reproductive outcomes after in vitro fertilization (IVF)?
Summary answer: There are no differences in reproductive outcomes if cryopreserved embryos are transferred after overnight culture or after two hours of culture after thawing
What is known already: There is currently no clear consensus on the optimal time for the thawing of embryos in freezing protocols, despite the fact that the identification of an adequate culture time would improve embryo culture environments allowing for better reproductive results
Study design, size, duration: A systematic review of studies evaluating the association of two different culture times after thawing, and before transfer, of cryopreserved embryos with the incidence of pregnancy, implantation, abortion, live birth or ongoing pregnancy, and ectopic pregnancy in individuals after IVF fertilization
Participants/materials, setting, methods: A literature search was performed in PubMed, EmBase, and the Cochrane library (from January 2000 to august 2019). A cumulative meta-analysis and evaluation of heterogeneity was performed for the clinical pregnancy rate. The quality of the included studies was assessed using Cochrane's Risk of Bias tool and ROBINS I for observational studies
Main results and the role of chance: Five studies were included, two were randomized controlled trials and three were retrospective published between 2010 and 2019. One study included exclusive transfers of day 5 embryos; four studies included only transfers of day 3 embryos. The clinical pregnancy rate had a high degree of heterogeneity and no difference was found between short and long embryo culture
Limitations, reasons for caution: One limitation was the quality of studies reported and included. Most were retrospective in design and only two were RCTs. The risk of bias was deficient, with moderate being the best quality. The small number of studies reported on this subject was a very important limitation
Wider implications of the findings: No differences were found if cryopreserved embryos were transferred after overnight culture or after two hours of culture after thawing. Large-scale randomized clinical trials should be conducted to definitively clarify if there is a difference in clinical outcomes after different culture time between thawing and transfer of cryopreserved embryos
Trial registration number: CRD42019137136

P-194 Feasibility of distance-based scoring in the Well-of-the-Well (WOW) culture system to improve blastocyst development

R. Minekawa[1], T. Yoshida[1], H. Ogata[1], S. Ogata[1]

[1]Ogata Family Clinic, Obstetrics and Gynecology, Hyogo, Japan

Study question: To provide quantitative assessment of optimal arrangement of embryos in the Well-of-the-Well (WOW) culture system to improve blastocyst development and quality.

Summary answer: Scorings that were based on numbers of surrounding embryos and distance between adjacent embryos were associated with blastocyst formation.

What is known already: It is known in bovine and mouse embryos that culturing embryos in groups is superior to single culture for blastocyst development due to secretion of autocrine and paracrine factors to neighboring embryos. The WOW system provides group culture environment in single drop of medium that could stimulate diffusion of autocrine and paracrine factors while observing individual embryo development simultaneously. It has been reported in human embryos that density may affect embryo quality in microwell group culture dish.

Study design, size, duration: This retrospective analysis of 476 embryos was conducted over a 24-month period between 2017 and 2019. Embryos originated from women <40 years old were enrolled in the study when nine and more embryos were obtained in each treatment cycle. The WOW system named LinKID®micro25 (DNP, Japan), which is consist of 5x5 microwells, were used. Embryos were cultured in single-step medium in LinKID®micro25 up to blastocyst stage without refreshment.

Participants/materials, setting, methods: Embryos were firstly compared between two groups according to number of adjacent embryos; Group A (1-4) and B (5-8) respectively. Secondly, considering different intra-microwell distance, cumulative scoring was set up as follows; 2 points were given when embryos were allocated side-to-side or up and down, and 1 point was given when embryos were situated diagonally. Then embryos were grouped based on total scores; Low (2-6), Intermediate (7-9), and High (10-12) points (P) groups.

Main results and the role of chance: Blastocyst formation rate and percentage of good quality blastocysts (≥3BB by Gardner grading scheme) were main outcome measures. In the first analysis the blastocyst formation rate was significantly higher in Group B (A: 50.3% vs B: 59.7%, P=0.048) but no differences were found in average age (A: 34.0 vs B: 34.2 years) and the percentage of good quality blastocyst (A: 67.0% vs B: 69.8%). In the second part of study, there was no difference among three groups in terms of average age; Low P (N=167) 34.1, Intermediate P (N=217) 34.0, High P (N=92) 34.6 years. Blastocyst formation rates were 50.3%, 56.7%, and 64.1% in Low P, Intermediate P and High P group respectively, and it was significantly higher in High P group compared to Low P group (P=0.037). The more points it gained, the higher percentage of good quality blastocyst (65.5% vs 69.9% vs 71.2%) was observed although there was no statistical significance.

Limitations, reasons for caution: This is a retrospective analysis of database, and sample size is limited as cases were restricted to patients obtained more than nine embryos for equalizing background conditions. Our study focused on blastocyst formation and quality, hence there is no enough data to evaluate pregnancy rate and birth outcomes.

Wider implications of the findings: This distance-based scoring approved possible autocrine/paracrine effect for human embryo development. It is the first quantitative method to propose optimal arrangement of embryos in the WOW culture, which could lead to better chance for clinical pregnancy. Cases with fewer embryos could also have benefit if included in the future study.

Trial registration number: Not applicable

P-195 Pulling vs Flicking. Does biopsy technique affect clinical outcomes on euploid embryos?

V. Montalvo[1], S. Novo[1], A. Garcia-Faura[1], B. Marques[1], F. Garcia[1], C. Castelló[1], M. López-Teijón[1]

[1]Institut Marques, Reproductive Medicine Service, Barcelona, Spain

Study question: Has the technique used during trophectoderm biopsy any effect on euploid embryos?

Summary answer: Pulling and Flicking techniques provide, when applied properly, optimal and similar results. Therefore, both techniques can be used indistinctly to perform trophectoderm biopsy.

What is known already: Embryo biopsy for Preimplantational Genetic Testing (PGT) has to accomplish two main goals: the biopsied material has to mirror the genetic constitution of the embryo, and embryo viability cannot be impacted. The strategy that comes closer to guarantee these two aspects is trophectoderm (TE) biopsy. Since there is no standardized TE biopsy procedure, two techniques are coexisting (Pulling and Flicking). The main difference between both techniques is how cells are detached from the embryo. Considering that TE biopsy could affect TE quality/integrity, hence implantation, studies are needed in order to evaluate the effect of different biopsy techniques

Study design, size, duration: This was a prospective observational study performed between 2018 and 2019. 286 PGT-A treatments with 990 TE biopsies were included. Of these, 145 euploid embryos were individually transferred, 84 biopsied using Pulling and 61 via Flicking. When using Pulling, laser pulses debilitate cell junctions and applying moderate tension the TE sample is detached. However, when using Flicking this tension is replaced by a cutting motion generated between the holding and biopsy pipettes.

Participants/materials, setting, methods: In every single embryo biopsy, the number (1-10) and intensity (ms) of laser pulses was registered, as well as the operator and the method used for the biopsy (Pulling or Flicking). Blastocyst area (μm^2) before and after TE biopsy as well as just before embryo vitrification was measured for each transferred embryo.

The main outcomes were implantation rates and embryo collapse-expansion dynamics.

Main results and the role of chance: No differences were found between groups regarding egg age (Pulling: 33.9 vs Flicking: 32.97; p=0.885), maternal age (Pulling: 39.69 vs Flicking: 39.68; p=0.94), PGT indication (p=0.422), embryo expansion (p=0.205) and quality (p=0.63), and laser intensity (Pulling: 416.4ms vs Flicking: 438.3ms; p=0.184). Otherwise, the number of laser pulses was higher when using pulling (Pulling: 5.4 pulses vs Flicking: 3.49 pulses; p<0.05). This difference was expected and intrinsically attributed to the technique. To ensure that this was not a confounding factor we analysed implantation rate based on the number of pulses and no significant differences were detected (1-3 pulses: 63.6%; 3-6 pulses: 58.1%; >6 pulses: 76.5%).

Clinical outcomes, such as implantation rate (Pulling: 65.0% vs Flicking: 58.2%), miscarriage rate (Pulling: 10.0% vs Flicking: 9.1%), and ongoing pregnancy rate (Pulling: 55.0% vs Flicking: 49.1%), were equivalent between groups (p>0.05). Regarding collapse-expansion dynamic, we found that embryos from Flicking group showed the highest collapse percentage ((μm^2 Pre-μm^2 Post)/μm^2 Pre) after biopsy (Pulling: 47.4% vs Flicking: 56.1%; p<0.05). Nevertheless, we did not found any difference in the speed of expansion (%expansion/hour) after biopsy (Pulling: 11.3%/h vs Flicking: 11.0%/h; p>0.05). Neither the percentage of collapse nor the speed of expansion seem to affect implantation rates (p=0.325).

Limitations, reasons for caution: These results are preliminary. In order to ensure the safety of both techniques more data should be analysed. If possible, the impact on live birth rates would be the ideal outcome to assess if any of these two techniques proves to be superior.

Wider implications of the findings: Our results show that the type of technique used to perform TE biopsy does not affect clinical outcomes on euploid embryos. Although Flicking proved to elicit a stronger contraction post biopsy, probably by generating more mechanical stress to the trophectoderm, this did not seem to impair reproductive potential.

Trial registration number: Not applicable

P-196 Impact of cell exclusion and extrusion during the peri-compaction period on blastocyst formation and subsequent live birth

K. Shimazaki[1], G. Coticchio[2], K. Ezoe[3], K. Ohata[3], M. Ninomiya[1], N. Wakabayashi[1], T. Okimura[1], K. Uchiyama[1], T. Kobayashi[4], C. Lagalla[2], A. Borini[5], K. Kato[4]

[1]Kato Ladies Clinic, IVF Laboratory, Tokyo, Japan ;
[2]9.Baby- Family and Fertility Center, IVF laboratory Unit, Bologna, Italy ;
[3]Kato Ladies Clinic, R&D Division, Tokyo, Japan ;
[4]Kato Ladies Clinic, Gynecology, Tokyo, Japan ;
[5]9.Baby- Family and Fertility Center, Reproductive Medicine IVF, Bologna, Italy

Study question: Are cell exclusion and extrusion during the embryo peri-compaction period associated with embryo development, pregnancy outcomes and patient characteristics after blastocyst transfer?

Summary answer: Cell exclusion and extrusion during peri-compaction are associated with impaired blastocyst expansion, reduced live birth rates, male age and low sperm-motility.

What is known already: The biological and clinical significance of morphological alterations in human morula stage embryos is poorly understood, in part because most assessments of morula morphology have been performed

statically. With the flexibility offered by time-lapse technology, different patterns have recently been identified in compacted embryos: fully-compacted morula (FCM), partially-compacted morula with cells excluded before compaction (Excluded-PCM), and partially-compacted morula with cells extruded after compaction (Extruded-PCM). Excluded-PCM and extruded PCM have been associated with decreased blastocyst formation, and increased degeneration rates, respectively. At present, our overall knowledge of possible associations between compaction patterns and pregnancy outcomes, and patient/embryonic characteristics is rather limited.

Study design, size, duration: Time-lapse videos from 384 morula stage embryos in 291 patients who underwent ICSI with minimal stimulation and single vitrified-warmed blastocyst transfer (SVBT) from April 2017 to March 2018 were retrospectively analysed. Compaction patterns were assessed and compared with embryonic and pregnancy outcomes after SVBT. Other factors possibly associated with compaction patterns, such as patient- and cycle-characteristics and early cleavage-stage development, were investigated.

Participants/materials, setting, methods: Following ICSI, time-lapse videos spanning fertilization to blastocyst stage were analysed. The number of cells excluded before compaction, and cells extruded after compaction (if any) were annotated. Possible correlations of compaction patterns with blastocyst expansion rates, blastocyst morphological grade, clinical and ongoing pregnancy rate, and live birth rate were evaluated. Other possible relationships between compaction patterns and patient characteristics, including serum hormone levels, semen parameters, and incidence of abnormal cleavage patterns were also assessed.

Main results and the role of chance: The incidences of FCM, Excluded-PCM, and Extruded-PCM were 51.8%, 29.7%, and 18.5%, respectively. Blastocyst formation and cryopreservation rates in Excluded-PCM and Extruded-PCM groups were significantly lower than that in the FCM group, particularly when the numbers of cells excluded and extruded were more than 3 and 2, respectively. Lower morphological grades of inner cell mass and trophectoderm were observed in both Excluded-PCM and Extruded-PCM groups compared with the FCM group. Cochran-Armitage trend testing demonstrated that the ongoing pregnancy and live birth rates were significantly lower in Excluded-PCM and Extruded-PCM groups than in the FCM group ($P = 0.0147$, $P = 0.0348$, respectively). Increases in incidence of cell exclusion and extrusion were significantly associated with increases in male age and decreases in sperm-motility ($P = 0.0108$, $P = 0.0019$, respectively). Furthermore, the embryos in the Excluded-PCM group exhibited significant correlations with higher incidence of direct cleavage, rapid cleavage, and asymmetrical division ($P = 0.0145$, $P < 0.0001$, $P = 0.0232$, respectively). Embryos in the Extruded-PCM group did not correlate with irregular division in early stage cleavage.

Limitations, reasons for caution: The number of analysed embryos is relatively limited; therefore, further studies are required to more reliably assess the clinical significance of these alternative patterns of compaction.

Wider implications of the findings: These results support the possibility that compaction patterns can serve as a predictive parameter for pregnancy outcomes. In addition, they shed new light on the phenomenology and importance of a poorly investigated stage of human preimplantation development.

Trial registration number: not applicable

P-197 The timing of the first cleavage of direct cleavage embryos was delayed compared to normal cleavage embryos.

H. Takahashi[1], R. Hirata[1], T. Habara[1], N. Hayashi[1]

[1]Okayama couple's clinic, laboratory, Okayama, Japan

Study question: Are the early dynamics of direct cleavage (DC) embryos different from normal cleavage embryos?

Summary answer: In DC embryos, blastocyst transfer led to live birth, but the timing of the first cleavage was delayed compared to normal cleavage embryos.

What is known already: DC embryos exhibit lower blastocyst formation rates. However, we previously reported that DC embryos exhibit a similar implantation potential to that of normal cleavage embryos if the blastocyst stage is reached, in which embryo selection for transfer is based on blastocyst grade and diameter. Prior reports suggest that the timing of the first cleavage is associated with embryo development and higher incidence of chromosomal abnormalities, but there are few reports regarding the timing of the first cleavage in DC embryos.

Study design, size, duration: This was a retrospective study of data acquired by time-lapse imaging. The study included 679 embryos from 353 IVF cycles in 286 patients that underwent intracytoplasmic sperm injection (ICSI) treatment for blastocyst cryopreservation between May 2018 and August 2019. A total of 373 blastocysts were cryopreserved, and of these, 190 were used for a single vitrified-thawed blastocyst transfer by December 2019. Embryo selection for transfer was based on blastocyst grade and diameter.

Participants/materials, setting, methods: Embryos with DC at the first cleavage were recorded as DC(+), and embryos with normal cleavage were recorded as DC(-). We compared the time from ICSI to the beginning of the first cleavage (t2), and the time from the beginning to the end of the first cleavage (d2) between groups. In addition, we compared implantation rates, miscarriage rates and live birth rates in single vitrified-thawed blastocyst transfer cycles between groups.

Main results and the role of chance: T2 and d2 of DC(+) embryos (27.9 hours and 5.5 hours, respectively) were significantly longer than those of DC(-) embryos (25.6 hours and 3.3 hours, respectively, p<.0001), even when the embryos developed into blastocysts on Day 5 (t2: 27.0 hours in DC(+) and 25.2 hours in DC(-), p<.0001, d2: 5.9 hours in DC(+) and 3.2 hours in DC(-), p<.0001). T2 of embryos that reached high-quality blastocysts or that were used in single vitrified-thawed blastocyst transfer were similar in DC(+) (25.7 hours and 26.1 hours, respectively) and DC(-) embryos (24.8 hours and 25.2 hours, respectively). However, d2 was significantly longer in DC(+) embryos (5.8 hours and 5.7 hours, respectively) compared to DC(-) embryos (3.0 hours and 3.1 hours, respectively, p<.0001). In addition, in DC(+) embryos, the rate of blastocyst formation on Day 5 was higher when t2 was <26 hours than when t2 was >=26 hours (80.3% vs. 22.9%, p<.0001). No significant differences were detected in implantation rates, pregnancy loss rates and live birth rates between DC(+) and DC(-) embryos (29.2% vs. 40.1%, p=0.303, 28.6% vs. 29.7%, p=0.390, 20.8% vs. 27.4%, p=0.494, respectively). Congenital abnormalities were not detected in either group.

Limitations, reasons for caution: Small sample size is the primary limitation. In this study, we focused only on the first cleavage, and therefore events after the second cleavage were not evaluated. Because chromosomal analysis is strictly prohibited in Japan, this study had no available data regarding the chromosomal ploidy of the blastocysts.

Wider implications of the findings: The time of the first cleavage was prolonged in DC embryos, which should be investigated in future studies. However, our results suggest that the timing of the beginning of the first cleavage could be a useful indicator for embryo selection when the only transferrable embryos are DC embryos.

Trial registration number: not applicable

P-198 Detailed quality assessment of direct cleavage embryos during the first division using a combined early cleavage with an embryo morphological grading method

H. Tomari[1], K. Honjo[1], Y. Nagata[1]

[1]IVF NAGATA Clinic, ART Lab, Fukuoka, Japan

Study question: Is it possible to perform a detailed quality assessment of direct cleavage (DC) embryos at the early embryonic stage (day2)?

Summary answer: The quality of DC embryos can be assessed in detail by combining the timing of the first cleavage and the morphological evaluation on day2.

What is known already: Several studies have reported a clear correlation between the occurrence of DC (divided into three or more cells) during the first division of an embryo and impaired embryo development potential in humans. In addition, it has also been reported that the pregnancy rate due to transfer of DC embryos that developed to blastocysts is similar to that of normal cleavage embryo transfer. However, only few studies have reported on methods for assessing DC embryo quality.

Study design, size, duration: This retrospective observational study was performed in a single *in vitro* fertilization (IVF) center. This study included patients undergoing IVF or intracytoplasmic sperm injection. We analyzed 1,242 DC embryos with normal fertilization using a time-lapse incubator. All study participants provided informed consent and the study design was approved by the

ethics committee of the IVF Nagata Clinic, Fukuoka, Japan. DC embryos were classified into 3- and ≥4-cell (4 cells or more) groups.
Participants/materials, setting, methods: Ex.1: We compared blastocyst formation rates between the two groups. Ex.2: The two groups were further classified into six grades by the following method. Embryos were evaluated for early cleavage (EC) or late cleavage (LC) at 27 hours after insemination and morphology was scored on day2 (poor, ≥4-cells with ≥50% frag.; fair, ≥4-cells with <50% and ≥20% frag.; good, ≥4-cells with <20% frag. and equal blastomere). The blastocyst formation rates of each group were compared.
Main results and the role of chance: Ex. 1: Among the 1,242 DC embryos, 669 were in the 3-cell group and 573 were in the ≥4-cell group. The blastocyst and high-quality blastocyst formation rates were significantly higher (p<0.01) in the 3-cell group than in the ≥4-cell group (53.5% vs. 32.7%, 28.0% vs. 14.1%, respectively). Ex. 2: Among the 669 embryos in the 3-cell group, 211 were in the EC-fair embryos, 141 were in the EC-poor embryos, 75 were in the LC-fair embryos, and 242 were in the LC-poor embryos. Among the 573 ≥4-cell group, 102 were in the EC-fair embryos, 127 were in the EC-poor embryos, 90 were in the LC-fair embryos, and 254 were in the LC-poor embryos. The blastomeres of DC embryos were unequal and no embryo was evaluated as good. The blastocyst and high-quality blastocyst formation rates were significantly higher (p<0.05) in the EC-fair embryos of the 3-cell group than in the other groups (71.6% vs. 18.5%–57.8%, 44.1% vs. 5.1%–33.3%, respectively).
Limitations, reasons for caution: This study lacked data about implantation rates after embryo transfer. To investigate the implantation ability of DC embryos, it is necessary to increase the number of cases where data were analyzed after embryo transfer.
Wider implications of the findings: The EC-fair embryos of the 3-cell group had high blastocyst development ability for DC embryos. The results suggest that the detailed evaluation of DC embryos at the early embryonic stage is not only predictive of embryogenic potential, but also useful for the selection of embryos for early embryo transfer.
Trial registration number: Not applicable

P-199 The impact of the oocyte donor's age on the recipient's outcomes: Should we exclude very young women from oocyte donation?

M. Roca Feliu[1], E. Clua[1], M. Tresánchez[1], S. García[1], N.P. Polyzos[1], F. Martínez[1]

[1]Hospital Universitari Quirón Dexeus, Obstetricia- Ginecología y Reproducción, Barcelona, Spain

Study question: Does the oocyte donor's age have an impact on the live birth rate (LBR) in the recipients?
Summary answer: The LBR is significantly lower when donors are younger than 25 years and, especially, when they are younger than 20 years.
What is known already: Donor's age becomes one determining factor for successful in oocyte donation programs (OD). It has been demonstrated that success is determined by embryo quality which depends on the oocyte quality which in turn depends on the donor's age. In most countries where OD is anonymous, law or guidelines establish that age must be between 18 and 35. Previous studies have shown that donor age < 25 was not associated with better outcomes. However, no studies have separately analysed LBR in very young donors (<20 years) despite previous reports suggesting a higher aneuploidy rate in women between 18 and 25 years.
Study design, size, duration: A retrospective study of 3766 oocyte reception cycles performed between January 2009 and December 2018 was carried out. Cycles using vitrified oocytes, PGD or TESE were excluded.
Participants/materials, setting, methods: The cycles were categorized considering the donors' age groups: < 20, 20-25 and ≥26 years. The statistical tests used were Chi-square test for evaluating the differences in LBR and ANOVA to test differences in fertilisation and embryo development rates as well as for testing embryo quality. A generalized linear mixed model was applied to estimate the odds for every endpoint. Patient was treated as random factor to avoid repeated observations effect.
Main results and the role of chance: A total of 3766 oocyte reception cycles were analysed. The pregnancy rate was 51.4% and the LBR was 40.7%. In 4.7% of the cycles the age of the donor was <20 years, in 41.1% was between 20-25 years and in 54.2% was ≥26 years. When we analysed LBR according the donors' age we found significant differences: 33.9% for the youngest group, 39.1% for the group of 20-25 years and 42.5% for donors over 25 years (p=0.022). When adjusting by confounding factors (recipient age, number of transferred embryos, and day of embryo transfer), LBR was lower to the <20 years' group (OR: 0.70; CI 95%: 0.50-0.99) and to the 20-25 years' group (OR: 0.85; CI 95%: 0.74-0.98) compared to the ≥26 years' group. No significant differences were observed neither in fertilisation rates (74.2%, 76.1% and 77.5%) nor in embryo development rates (57%, 61.4% and 62%). The number of good-quality embryos transferred was significantly lower in the <20 years' group (1.03±0.71; 1.18±0.69; 1.19±0.67; p=0.015).
Limitations, reasons for caution: The group of donors <20 years included in our study was relatively small and smaller as compared with the groups of 20-25 years and ≥26 years. Nonetheless, the sample size had adequate statistical power in order to identify significant differences in live birth rate.
Wider implications of the findings: Oocyte donor age <20 years as compared with older oocyte donors is associated with significantly lower live birth rates among oocyte recipients. Further studies are needed to confirm our findings, in order to determine whether in the future oocyte donation programs should also have a lower age limit.
Trial registration number: -

P-200 Blastocyst utilization rate – is it a matter of culture media?

M. Schenk[1], N. Reinschissler[2], C. Fritz[3], M. Hörmann-Kröpfl[3], G. Weiss[4]

[1]Das Kinderwunsch Institut Schenk GmbH, Research & Development, Dobl, Austria ;
[2]Das Kinderwunsch Institut Schenk GmbH, Research and Development, Dobl, Austria ;
[3]Das Kinderwunsch Institut Schenk GmbH, Embryology, Dobl, Austria ;
[4]Das Kinderwunsch Institut Schenk GmbH, Research and Development, Dobl bei Graz, Austria

Study question: Is there a difference in blastocyst formation rates in embryos cultured with G-TL™ (*Vitrolife*) versus GM501 (*Gynemed*)?
Summary answer: G-TL™ reveals a higher blastocyst formation rate compared to GM501.
What is known already: A variety of studies have been carried out to compare the effects of different culture media types on embryo development, showing controversial results. A recent Cochrane review suggested that available data are insufficient to conclude the best culture medium for embryo quality, pregnancy and implantation. Besides the use of the medium, the day of embryo transfer is also under debate. A current meta-analysis showed no differences in reproductive outcomes comparing blastocyst (d5) and cleavage-stage (d3) embryo transfer in clinical practice, however, the trend for a blastocyst transfer is increasing since it resembles the natural process of implantation.
Study design, size, duration: The prospective study included 641 embryos from patients undergoing IVF (in vitro fertilization) and ICSI (intracytoplasmic sperm injection) treatment. Patients with ≥ 6 MII oocytes were included in the study. Oocytes were splitted equally into the two media types GM501 and G-TL™, both overlaid with mineral-oil (Gynemed).
Participants/materials, setting, methods: The study was designed and conducted at the Kinderwunsch Institut Schenk GmbH (Dobl, Austria) in 2019. Fertilized occytes from women aged 18 - 41, cultured in Embryoscope time-lapse system (Vitrolife) were included while oocytes/embryos with polar body biopsy/trophectoderm biopsy were excluded. The utilization rates of day 3 and day 5 embryos were analyzed.
Main results and the role of chance: The number of achieved fertilized oocytes was 308 in GM501 media and 333 with G-TL™, respectively. Overall the utilization rate was 45.7% using G-TL™ media compared to 36% in the GM501 group. A subgroup analysis of day 3 revealed a utilization rate of 13.6% with GM501, compared to 10.5% in the G-TL™ group. Additionally, the utilization rate of day 5 embryos was 22.4% using GM501 versus 35.1% with G-TL™.
Limitations, reasons for caution: The sample size may be seen as study limitation. However, statistically significant results were obtained. Nonetheless, results should be confirmed with a higher sample size.
Wider implications of the findings: The data show a significant better rate of blastocyst utilization with G-TL™ media. Since around 5 days after fertilization

the uterus is particularly receptive, it is tempting to speculate that transferring blastocysts in general is more favorable for a successful treatment outcome.
Trial registration number: Not Applicable

P-201 supplementation of culture medium with melatonin improves blastocyst development and reverses glucose intolerance in IVF mice

Y. Jia[1], W. Liu[1], D. Bai[1], K. Li[1], M. Chen[1]

[1]Center for Reproductive Medicine- Shanghai First Maternity and Infant Hospital, Tongji University, Shanghai, China

Study question: Whether the adverse effects of in vitro fertilization (IVF) on glucose metabolism could be reversed by supplementation of culture medium with melatonin in mice?
Summary answer: Supplementation of culture medium with melatonin improved IVF blastocyst differentiation, reduced excessive weight gain after birth and normalized glucose intolerance in IVF male mice.
What is known already: Approximately more than 8 million children have been conceived by assisted reproductive technologies worldwide. Increasing evidence suggests that IVF treatment may be associated with an increased risk of developing obesity and metabolic diseases in adulthood. IVF mouse models confirmed adult IVF offspring showed excessive weight gain on a high fat diet, impaired glucose metabolism and cardiovascular dysfunction compared with naturally conceived mice. Notably, in vitro culture impaired blastocyst developmental kinetics and differentiation, independently of ovarian stimulation. Moreover, it has been shown that addition of melatonin to culture medium may improve embryo development and prevent cardiovascular alterations in IVF mice.
Study design, size, duration: A cross-sectional mouse model was utilized, wherein blastocysts were generated by natural mating (control group) or IVF with or without melatonin (10^{-6}M) (mIVF and IVF group respectively) in clinical grade fertilization and culture media. Blastocysts were transferred to pseudo-pregnant ICR females. Cell lineage allocation was assessed for 30-40 blastocysts from each group. Body weight, glucose tolerance, energy expenditure, hepatic gene expression was measured in 6-10 mice from each group.
Participants/materials, setting, methods: C57BL/6 females and DBA2 males were used to generate blastocysts. Cell numbers of inner cell mass (SOX2) or trophectoderm (Cdx2+) were determined by immunohistochemistry. Males were weaned at 3 weeks of age onto a chow diet or a high-fat diet (60% fat) for 8 weeks. Glucose tolerance was assessed by an intraperitoneal glucose tolerance test (2g/Kg). Energy metabolism was examined in metabolic cages. Hepatic gene expression was measured by RNA-Seq and validated by qPCR.
Main results and the role of chance: Blastocyst rates were similar in the two IVF groups. Reduced inner cell mass, trophectoderm and total cell number were observed in IVF blastocysts, compared with the control group ($P<0.05$). IVF pups had significantly lower birth weight and body weight before weaning, but exhibited increased body weight and liver weight at 11 weeks of age compared with controls ($P<0.05$), independently of diet. IVF mice were glucose intolerant shown as an increased glucose area under the curve, and had decreased energy expenditure as well as a large number of differentially expressed genes related to metabolic pathways in the liver tissue compared with the control group ($P<0.05$), independently of diet. The mIVF group showed intermediate cell numbers in blastocysts, body weight, liver weight and differentially expressed hepatic genes compared with the IVF group and control group, and normalized glucose tolerance and energy expenditure compared with the IVF group ($P<0.05$), independently of diet. Importantly, there was no significant difference in glucose tolerance and energy expenditure between the mIVF group and the control group.
Limitations, reasons for caution: The findings in C57BL/6J x DBA2 F1 mice may not represent human infertility which is complex and often multi-factorial. Further studies are needed to confirm these findings in human settings and investigate the underlying mechanisms by which melatonin improves embryonic development and adult metabolic phenotypes.
Wider implications of the findings: The data shows that melatonin mitigated the detrimental effects of in vitro fertilization and culture on mouse embryo development and catch-up growth after birth, and reversed glucose intolerant in adult male offspring. Therefore supplementation of culture medium with melatonin may be beneficial for human IVF.
Trial registration number: not applicable

P-202 Synchronizing blastocyst at hatching status in frozen embryo transfer cycles: A randomized controlled trial

Y. Magdi Abd-Elkreem[1], M.H. Zidan[2], M.A. Ibrahim[3], Y. Edris[3], A. El-Gohary[3], A. Elattar[4]

[1]Al-Yasmeen Fertility and Gynecology Centerv, IVF Laboratory, Banha, Egypt ; [2]Gannah IVF Hospital, IVF Lab. director, Cairo, Egypt ; [3]Faculty of Medicine- Benha University, Department of Obstetrics and Gynecology, Benha, Egypt ; [4]Al-Yasmeen Fertility and Gynecology center, . Department of Obstetrics and Gynecology, Benha, Egypt

Study question: Is culture of vitrified-warmed blastocysts for another day after warming and selection of only blastocysts that could reach hatching status associated with improved outcomes?
Summary answer: Culture blastocyst with ≤ 3grade of expansion for another day after warming may be associated with improved implantation and ongoing implantation rates.
What is known already: Failure of blastocyst hatching has been identified as a potential obstacle limiting human implantation efficiency. On the other hand, discordance between the stage of transferred blastocyst and the receptive endometrium. We aimed in the present study to identify whether transferring frozen/thawed blastocysts at hatching status could improve the ongoing implantation rate in case of adjusting the thawing time to achieve this stage at the transfer day.
Study design, size, duration: This randomized controlled trial was conducted at two private ART centers in Egypt, and involved cycles of vitrified-warmed blastocyst transfer after ICSI. Recruitment of first participant was on April 25, 2017, and the last participant was on May 10, 2018. A total of 469 women undergoing frozen embryo transfer (FET) were randomized into two groups.
Participants/materials, setting, methods: Randomization was performed 2 days prior to a scheduled FET. Participants received FET either with hatching or hatched blastocysts achieved by incubation of ≤ 3 grade of expansion blastocysts after thawing 18-24 hours (study group, n=232) or with ≤ 3 grade of expansion blastocysts incubated only 2-4 hours (control group, n=237).
Main results and the role of chance: Baseline and treatment characteristics of the study population were balanced for the two study groups**.**
The implantation rate (45.6% vs. 38.2%; OR 1.35, 95% CI 1.03 to 1.78) and ongoing implantation rate (38.2% vs. 30.5%; OR 1.4; 95% CI, 1.06 to 1.87) were statistically significantly improved in the study group compared with the control group. There was no significant difference in the rates of livebirth (47.4% vs. 38.8%; OR 1.42, 95% CI, 0.98 to 2.05), clinical pregnancy (56% vs. 50.2%; OR1.26, 95% CI, 0.88 to 1.82), ongoing pregnancy (50% vs. 42.6%; OR 1.35, CI, 0.94 to 1.94), and miscarriage (5.6% vs. 5.9%; OR 0.95, 95% CI, 0.44 to 2.06) in the study group compared with the control group. The multiple pregnancy rate was similar in the study group (62/232; 26.7%) compared with the control group (48/237; 20.3%; OR 1.44; 95% CI, 0.93 to 2.21; P=0.1).
Limitations, reasons for caution: The age of the study population was relatively young, thus we should also be cautious to extend the results to older patients. Further large scale multicenter randomized controlled studies are required to confirm our findings.
Wider implications of the findings: Prolonged culture of blastocysts with ≤3grade of expansion may not only provide better embryo–endometrium synchrony, but also induces self-selection of the most viable blastocysts which have the capacity to resume its development.
Trial registration number: NCT03128970

P-203 Successful pregnancies following transfer of blastocysts derived from delayed-intracytoplasmic sperm injection (delayed-ICSI)

J.P. Sam[1], A.Y.X. Lim[1], Z.Q. Tee[1], C.S.S. Lee[2]

[1]Alpha IVF & Women's Specialists, IVF laboratory, Petaling Jaya, Malaysia ; [2]Alpha IVF & Women's Specialists, Medical Director, Petaling Jaya, Malaysia

Study question: To evaluate the clinical outcome of delayed-ICSI on day1-matured oocytes.
Summary answer: Blastocysts derived from day1-matured oocytes can result in successful pregnancies and thus can be considered for transfer when there is no blastocyst available from day0-ICSI.

What is known already: Delayed-ICSI is defined as the ICSI procedure performed on oocytes matured via extended culture (day1-matured oocytes). It is a routine procedure in Alpha IVF & Women's Specialists in order to improve cycle outcomes of selected patients, especially poor responders.
Despite the lower fertilisation, blastulation and blastocyst utilization rates of delayed-ICSI compared to day0-ICSI, the pre-implantation genetic testing for aneuploidies (PGT-A) revealed that delayed-ICSI blastocysts can be chromosomally normal (euploid) and can therefore be considered for embryo transfer (Lee, C.S.S., 2018).

This abstract reports the successful pregnancies following the transfer of delayed-ICSI blastocysts.

Study design, size, duration: A total of 370 IVF treatments at Alpha IVF & Women's Specialists between January 2018 and December 2019 had delayed-ICSI done on their day1-matured oocytes (mean maternal age: 37.6; range 23.0-47.0). Indications for a delayed-ICSI includes immature oocytes retrieved on day-0 (*n*=50); fail or abnormal fertilization on day0-ICSI (*n*=36); poor fertilization on day0-ICSI (<50%) (*n*=11); low oocyte maturation rates (<50%) (*n*=8); and poor responders (≤6 oocytes injected for day0-ICSI) (*n*=265).

Participants/materials, setting, methods: The immature oocytes were cultured in incubators for 18-24 hours and delayed-ICSI (PIEZO, Japan) was performed on oocytes that were mature on day-1. Injected oocytes were cultured up to 7 days and utilisable blastocysts were vitrified (Cryotec, Japan) on Day5, Day6 and/or Day7 with/without trophectoderm biopsy. Cells biopsied were amplified and screened using Next Generation Sequencing (NGS) (IonTorrent, USA). Selected blastocysts were thawed for frozen single blastocyst transfer (SBT) and the clinical outcome was analysed.

Main results and the role of chance: Delayed-ICSI was done on 980 day1-matured oocytes, of which 644 were 2PN (65.7%), 122 were >2PN (12.5%), 136 were <2PN (13.9%) and 78 were degenerated (8.0%). The blastulation and utilisation rates (per 2PN) were 36.5% (235/644) and 19.9% (128/644) respectively. Of the 128 utilisable blastocysts, 108 blastocysts had PGT-A screening and 26.9% (29/108) were chromosomally normal (euploid). At the time of writing, a total of 12 delayed-ICSI blastocysts (10 euploid, 1 mosaic and 1 untested) were thawed (post-thaw survival rate=100%) for frozen SBTs as no suitable day0-derived blastocysts were available for transfer (mean age: 36.0; range:31.0-42.0). Nine (9) had positive β-HCG reading and gestational sacs were seen in all nine patients (clinical pregnancy rate = 75.0%; implantation rate = 75.0%). Seven (7) patients have ongoing pregnancies ranging from 11-36 weeks whereas 2 patients were known to have miscarried at the 8th and 11th week of pregnancy.

Limitations, reasons for caution: The immature oocytes were further cultured up to 18-24hours *in-vitro*. The long incubation duration may result in post-mature oocytes that could affect blastocyst development. Further studies could employ other maturity assessment tools such as spindle visualization prior to delayed-ICSI, rather than having a fixed incubation duration to optimise delayed-ICSI outcomes.

Wider implications of the findings: Good pregnancy and implantation rates can be obtained with blastocysts derived from delayed-ICSI. Therefore, patients who are unlikely to have a good number of normally fertilized oocytes on day-0 should have their immature oocytes cultured to day-1 for delayed-ICSI to generate more utilisable blastocysts.

Trial registration number: Not Applicable

P-204 Impact of maternal age and in vitro maturation on the transcriptome of denuded human oocytes.

S. Llonch[1], M. Barragán[2], P. Nieto[3], A. Mallol[1], M. Elosua[3], S. Ruiz[3], F. Zambelli[2], H. Heyn[3], B. Payer[1], R. Vassena[2]

[1]Center for Genomic Regulation- CRG, Gene Regulation Stem Cells and Cancer, Barcelona, Spain ;
[2]Clínica EUGIN, Research and Development, Barcelona, Spain ;
[3]National Center for Genomic Analysis- CNAG, Single Cell Genomics Team, Barcelona, Spain

Study question: Does maternal age affect the transcriptome of human oocytes at the germinal vesicle (GV) stage or at metaphase II (IVM-MII) after maturation *in vitro*?

Summary answer: Oocytes' gene expression is predominantly affected by maturation stage and less by maternal age.

What is known already: Female fertility is inversely correlated to maternal age due to a depletion of the oocyte pool and a reduction in oocyte developmental competence. Few studies have addressed the effect of maternal age on the mature oocyte (MII) transcriptome, which is established during oocyte maturation. However, reduced sample numbers have been analysed, and the bias represented by distinct genetic backgrounds was difficult to control for. Here, we characterise and compare the transcriptional patterns of a large cohort of fully grown GV and IVM-MII oocytes from women of varying reproductive age, age can be considered as a continuous variable.

Study design, size, duration: In this basic research study 38 women were recruited from May 2018 to June 2019. The mean woman age was 28.8 years (SD=7.6, range 18-43). A total of 82 immature GV oocytes were included in the study as: GV (n=40), IVM-MII (n=32) or GV unable to mature after *in vitro* maturation (n=10). Their transcriptome was compared by single-oocyte RNA-seq analysis.

Participants/materials, setting, methods: Oocytes were collected either as GV or after *in vitro* maturation for 30 hours in G2^{TM} medium, and individually frozen in lysis buffer until processed according to Smart-Seq2 single-cell RNA-seq protocol. The RNA-seq data obtained was analysed using the Seurat R package, which has been recently developed for the quality control, analysis and exploration of single-cell RNA-seq data.

Main results and the role of chance: Data quality control was applied and samples containing >30% of mitochondrial DNA and less than 1,000 genes were filtered out, leaving 75 oocytes for further analysis. Preliminary exploration of the RNA-seq data using an algorithm for dimensionality reduction identified two clusters of oocytes according to their maturation stage (GV and IVM-MII) with 10430 and 8744 detected genes, respectively; and independently of maternal age. To identify differentially expressed genes according to maternal age, we set a cut-off at 35 years old, which is the accepted clinical definition of advanced maternal age. Gene expression was analysed in GV and IVM-MII comparing both age's groups (<35 vs. ≥35 years old); however, in our preliminary analysis, no candidate gene has been identified. Taking advantage of our dataset, which contains oocytes representing a continuous age range, we looked for genes that would show gradual change with age. Here, we considered the set of genes that do not change their expression due to maturation stage (i.e. those which would be necessary to provide developmental competence to the oocyte) and we independently analysed both, GV and IVM-MII. Although we found some transcripts with a tendency to increase or decrease according to age, a deeper analysis is required.

Limitations, reasons for caution: GVs were collected after ovarian stimulation and MII were *in vitro* matured. Therefore, their transcriptome might not represent the *in vivo* GVs or MIIs.
Non-polyadenylated transcripts, which regulate gene expression and could be responsible for impaired oocyte developmental potential with age, are not detected with our sequencing method.

Wider implications of the findings: Our analysis suggests that advanced maternal age does not strikingly affect oocyte gene expression at GV or IVM-MII stages. Nonetheless, some genes showed a tendency to change their expression with age. A deeper analysis is required to discriminate whether those changes have clinically relevant effects on reproductive outcomes.

Trial registration number: not applicable

P-205 Non-invasive metabolomics analysis of spent culture media predicts embryo viability.

S. Cabello[1], H. Abdulla[2], M.L. Seth-Smith[1], M. Escriba[3], J. Crespo[3], S. Munné[1], J.A. Horcajadas[1]

[1]Overture Life SL, Scientific department, Madrid, Spain ;
[2]Texas A&M University Corpus Christi, Chemistry, Corpus Christi, U.S.A. ;
[3]Juana Crespo Clinic, Embryology, Valencia, Spain

Study question: Are there metabolites in the spent media that can act as biomarkers to predict embryo implantation?

Summary answer: A set of culture-media specific biomarkers were identified that highly predicted implantation potential in several fertility centers.

What is known already: A critical step in IVF cycles is the selection of the best embryo to be transferred, since treatment success strongly depends on this choice. The standard of care tool for embryo selection has been morphokinetics criteria, which has low predictability to ascertain implantation potential. The effectiveness of ART remains limited and only 10%-30% of embryos

replaced in the uterus implant. During the last 20 years, the alternative has been PGT (Preimplantation Genetic Testing), an invasive method that requires embryo biopsy. For this reason, novel non-invasive embryo screening methods are required to optimize embryo selection using AI-morphokinetics, metabolomics and non-invasive PGT.

Study design, size, duration: This retrospective study was done on 197 samples collected in three different clinics, using two different culture media (Sage n=129, and Vitrolife n=68), over a period of two years. Sixty-nine of the spent media samples came from embryos that implanted (P) and 128 from non-implanted embryos (NP). Embryos were obtained and cultured using routine IVF practices. 20 - 40 µL of spent media were collected after incubation between days 3 and 5.

Participants/materials, setting, methods: Patients undergoing infertility treatment in 3 clinics were included in this study. Spent media was frozen at -20°C after collection. Metabolites were extracted from the spent media samples (20 µL) using an ultrafiltering approach to remove molecules >3KDa and then run in a UPLC-Fusion Orbitrap MS/MS system which determined the abundance of metabolites in each sample. Different statistical techniques were applied to reduce the huge number of metabolites found to the most informative ones.

Main results and the role of chance: More than 5,550 metabolites were identified and measured.

A first analysis was performed with 129 Sage media samples -30 from pregnant (P) and 99 from non-pregnant cycles (NP)-. A Sage-specific MPI (Metabolite Pregnancy Index) was built with the most informative metabolites to determine pregnancy potential. The non-supervised analysis (not telling the software which sample is pregnant and which is not) identified 100% for P and 63% of for NP. Using supervised analysis, 100% of P samples were identified and 81% of NP samples.

A second analysis was performed with 68 Vitrolife media samples from two different clinics. With the data from the first clinic samples (n=37: 20 P and 17 NP), a Vitrolife-specific MPI (Metabolite Pregnancy Index) was built with the most informative metabolites to determine pregnancy potential. For the first clinic, the analysis showed an ability to predict embryo viability of 100% for P and 88% for NP samples. A blind analysis with a third batch of Vitrolife samples from a second center (n=31: 19P+12NP) showed 78% of P samples were identified and 61% of NP samples.

Limitations, reasons for caution: The study was retrospective, but for one of the centers it was blinded and still yielded high implantation predictability. Nevertheless, a prospective clinical trial is underway. A small limitation of the test is that each culture media has a different subset of informative biomarkers. Other media are being validated.

Wider implications of the findings: This metabolomics test is non-invasive, inexpensive, does not required any change in embryology protocol, and can be combined with NI-PGT and or morphokinetics, having the potential in itself or in combination with other methods to significantly improve embryo selection without causing embryo damage.

Trial registration number: NA

P-206 Differential expression of microRNAs associated with apoptosis and implantation in human in vitro-produced blastocysts following re-vitrification

M. Daneshvar[1], M. Movahedin[1], M. Salehi[2], M. Noruzinia[3]

[1]Tarbiat Modares University, Department of Anatomical Sciences- Faculty of Medical Sciences, Tehran, Iran ;
[2]Shahid Beheshti University of Medical Sciences, Cellular and Molecular Biology Research Center, Tehran, Iran ;
[3]Tarbiat Modares University, Department of Medical Genetics- Faculty of Medical Sciences, Tehran, Iran

Study question: Does re-vitrification affect on the expression of microRNAs involved in apoptosis and implantation of human in vitro-produced blastocysts?

Summary answer: re-vitrification could change the expression of microRNAs involved in apoptosis and implantation of human in vitro-produced blastocysts

What is known already: Numerous studies have been carried out about the effect of vitrification on genetic and epigenetic changes in human embryos, and nowadays Embryo freezing is among useful and safe infertility treatment techniques. Also re-vitrification of the same embryos after thawing has both theoretical and applied cryobiological implications. sometimes, due to the embryologist's discretion, re-vitrification can be applied for good quality supernumerary thawed/warmed embryos that have not been transferred in the present cycle. However, there is almost no study about the effect of re-vitrification on genetic and epigenetics profile in human embryos.

Study design, size, duration: A total of 30 ICSI-derived human embryos were used to evaluate the effect of re-vitrification on miRNAs expression with real-time PCR and DNA fragmentation with TUNEL assay. Embryos were donated from fertile couples referring for family balancing program. Donated embryos were cultured to blastocyst stage and high quality blastocyst (AA-AB) assigned to three groups: fresh, vitrified and re-vitrified. Scoring of blastocysts were according to Gardner et al. (2012) grading system. embryos were collected from 2017-2019.

Participants/materials, setting, methods: Collected Embryos were vitrified on Cryotech carriers, with the method described by Kuwayama. After warming of blastocysts and 4 hours culturing of them, embryos were used individually for Simultaneous RNA extraction and cDNA synthesis. The relative quantification of miRna expression and their target genes expression was carried out by real-time PCR. TUNEL assessment was done using of an in situ cell death detection kit TMR red (Roche, Mannheim, Germany) based on the company's instructions

Main results and the role of chance: The results of this research showed that the re-vitrification of human blastocysts did not affect the ability of their re-expand in culture. A significant decrease in miR-16 and miR-let7a expression in re-vitrified and vitrified group was observed compared to the fresh ($p<0.05$) one. On the other hand, a significant upregulation of the target genes integrin β-3 and BCL2 in the re-vitrified and vitrified embryos was observed comparing with the fresh group ($p<0.05$). The expression of BAX as a pro-apoptotic gene gene showed a significant decrease in re-vitrification group comparing with the fresh one ($P<0.05$). In TUNEL assessment, there was no significance difference in the vitrification and re-vitrification groups compared to the fresh respect to the total number cells and also the apoptotic cell rate ($P > 0.05$)

Limitations, reasons for caution: Clinical investigations are necessary to evaluate the responses of embryos belonging to sub-fertile/infertile couples which may have less resistance potential to stressful situations and show a different response to re-vitrification.

Wider implications of the findings: This study showed that the re-vitrification of embryos changes the expression of genes involved in embryo survival and implantation. therefore, it seems the embryos with high-quality show adaptability and resistance toward stress which this response can include increased expression of anti-apoptotic proteins and genes involved in apoptosis and implantation.

Trial registration number: not applicable

P-207 Interaction of the hippo signaling pathway during bovine follicle activation and growth in vitro

N. Donfack[1], M. Devos[1], P. Vidal[1], I. Demeestere[2]

[1]Research Laboratory on Human Reproduction, Université Libre de Bruxelles, Bruxelles, Belgium ;
[2]Research Laboratory on Human Reproduction, Université Libre de Bruxelles and Obstetrics and Gynaecology- Erasme Hospital, Bruxelles, Belgium

Study question: How does biochemical inhibition of the Hippo pathway affect the activation and the growth of primordial follicles *in vitro*?

Summary answer: MAPK/ERK signaling pathway seems to promote follicles activation during *in vitro* culture independently of the hippo pathway.

What is known already: The Hippo pathway has been identified as the major suppressor of tissue overgrowth. It plays a crucial role in follicular activation in a non-physiological condition. Recent studies showed that hippo disturbance takes place during the tissue preparation and that, fragmentation-induced follicle growth is partially blocked by verteporfin.

Study design, size, duration: Bovine ovarian tissue fragments were exposed to a control medium containing 0.1% of DMSO or 3 µM of Verteporfin, a pharmacological inhibitor of YAP, *in vitro* for 3 or 24 h, and cultured for additional 3 days.

Participants/materials, setting, methods: Bovine ovaries were obtained from a slaughterhouse. The ovarian fragments were analyzed at Days 0, 3h or 3 days of culture. Cortex was either directly processed for immunohistological or western blot analysis (AKT, p-AKT, p-ERK1/2, RPS6, p-RPS6) or subjected to follicular isolation for assessment of gene expression (CCN2, BIRC1, Kit Ligand, Last 1 et 2). The follicle number and stage were evaluated to assess early follicular development. Survival was evaluated using TUNEL.

Main results and the role of chance: At least, *in vitro* culture for three days promoted follicular activation independently of treatment, triggered by the upregulation of p-ERK. However, the tissue fragments exposed to verteporfin for 3 h presented lower follicular activation than control. As observed in other species, p-AKT and p-RPS6 decreased throughout the culture period (these results will be confirmed by the ongoing PCR and immunohistochemistry). Moreover, verteporfin seems to not affect follicle survival when compared to control.
Limitations, reasons for caution: Transport and impact of *in vitro* culture may cover up the potential benefit of the Verteporfin on follicle activation.
Wider implications of the findings: Our results showed that exposure to verteporfin during fragmentation partially blocked follicular activation and suggested the contribution of MAPK/ERK signaling pathway on follicular activation during *in vitro* culture.
Trial registration number: not applicable

P-208 Extended time from second polar body extrusion to two pronuclei formation is related to aneuploid embryo formation

T.B. Hsieh[1], P. Chang[2], J. Meriano[2], R.F. Casper[2]

[1]*Wayne State University, Obstetrics & Gynaecology, Detroit, U.S.A. ;*
[2]*TRIO Fertility- University of Toronto, Obstetrics & Gynaecology, Toronto, Canada*

Study question: Can morphokinetic parameters be used to predict embryo aneuploidy?
Summary answer: An extended time period from the second polar body formation (2PB) to two pronuclei formation (PNa) is associated with an increased chance of aneuploid embryo.
What is known already: Preimplantation genetic testing for aneuploid (PGT-a) is a useful technique to enhance the implantation rate and decreases the miscarriage rate, particularly in advanced female population. Without question, PGT-a is an invasive procedure with potential harmful effects to the fetus. Time lapse microscopy (TLM) provides valuable morphokinetic parameters or morphological criteria for selecting better developed embryos to be transferred to improve implantation rate. However, current parameters still are not accurate enough to predict embryo ploidy or implantation potential.
Study design, size, duration: This is single center, retrospective cohort study conducted in a university affiliated fertility institute. A total of 1515 biopsied blastocysts were examined from 255 intracytosplasmic sperm injection (ICSI) cycles (female age, 23-48; average 37.64.1). We collected embryo records with complete TLM recording from the time of ICSI until blastocyst stage and a day 5/6 trophectoderm biopsy result for analysis. PGT-a results were obtained by using either array comparative genomic hybridization or next generation sequencing.
Participants/materials, setting, methods: Morphokinetic parameters includes tPB2(from insemination to 2PB), tPNa (from insemination to 2PN, tPNf (from insemination to pronuclei faded), T2-8 (from insemination to 2 to 8 cells), tM (from insemination to morula), tSB (from insemination to blastulation), tB (from insemination to full blastocyst), tEB (from insemination to expanded blastocyst), CC2 (from 2 to 3 cells), and CC3 (from 3 to 5 cells). Morphokinetic parameters were analyzed with Mann-Whitney-U test. *P*-values less than 0.01 were designated significant.
Main results and the role of chance: Of the total 1515 embryos, 1153 embryos were reported euploid and 362 of them were aneuploid embryos with an embryo aneuploid rate of 31.4%. 456 of the embryos also underwent TLM examination. 317 of these embryos were reported euploid and 139 of them aneuploid by PGT-a with embryo aneuploid rate of 43.8%. From the original Morphokinetic parameters, we further calculated time from 2PB to PNa (2PB-PNa), PNa to PNf, and PNf to T2 as new parameters to compare with the PGT-a results. The analysis of morphokinetic parameters and embryo ploidy status showed that PB2-PNa were correlated with an increased rate of aneuploid embryos. Aneuploid embryos have significantly longer time from 2PB to PNa (euploid 2PB-PNa: 4.2 1.8 hours, aneuploid: 5.0 2.18 hours). The other parameters didn't show any statistically significant differences.
Limitations, reasons for caution: The data variation is wide. These data are single center, sample size of this study is small and it is a retrospective study. In addition, more scientific and strict criteria to define 2PB, PNa, and PNf may be able to be established.
Wider implications of the findings: Our finding suggested that the time needed for embryos to develop from 2PB to PNf may be used as a predictor for aneuploid embryo.
Trial registration number: not applicable

P-209 Embryo morphokinetics and static morphology in the prediction of live birth: evidences that speed is more important than beauty.

A. Bartolacci[1], C. Moutier[1], D. Turchi[1], M. Lain[1], E. De Ponti[2], M. Mignini Renzini[1], J. Buratini[1,3], M. Dal Canto[1]

[1]*Biogenesi - Reproductive Medicine Centre, Istituti Clinici Zucchi, Monza, Italy ;*
[2]*ASST Monza, Department of Medical Physics, Monza, Italy ;*
[3]*Sao Paulo State University, Institute of Biosciences, Department of Physiology*

Study question: Is embryo morphokinetics more accurate than conventional embryo morphology in the prediction of live birth following day 3 fresh embryo transfer?
Summary answer: Embryo morphokinetics was more accurate than conventional embryo morphology in ranking embryos in terms of live birth potential following a day 3 fresh embryo-transfer.
What is known already: IVF/ICSI success critically depends on the accuracy of embryo selection, which has been traditionally performed with the utilization of subjective and static morphological criteria. Time-lapse technology has recently allowed a dynamic morphological evaluation, and, through the utilization of several morphokinetic parameters, the comparison of embryos'developmental performance aiming for more accurate embryo selection and higher chances of a live birth. Indeed, this technology has been already reported to increase accuracy of embryo selection, with positive impacts on pregnancy rates. Nevertheless, most IVF clinics still perform embryo selection based on static morphological criteria.
Study design, size, duration: This is a retrospective study including patients in their first ICSI cycle with embryo culture in a time-lapse system, performed from January 2015 to June 2018. 828 embryos from 461 fresh embryo transfers (SET: n= 156; DET: n=305) were included in this study, in which each individual embryo was evaluated for both morphological and morphokinetic quality. Patients giving birth to a single child after a double embryo-transfer were excluded from the analysis.
Participants/materials, setting, methods: The primary end-point was live birth. A generalized estimation equation was utilized to control for dependencies within individuals, since some patients received two embryos at transfer. The performance characteristics of the morphokinetics model and conventional embryo morphology was calculated using a ROC curve. Nine subgroups were created crossing three morphology degrees (A, B, C) with three morphokinetics degrees (A, B, C) attributed to each embryo, and live-birth rates were compared between these subgroups.
Main results and the role of chance: For each morphokinetic parameter, an optimal range was defined combining the two quartiles presenting the highest live-birth rate. The parameters to be inserted in the model were selected by logistic regression, and were: t4 [OR = 2.04 (95% CI 0.87 – 4.77)] and t2 – tPNf [OR = 1.52 (95% CI 0.99 – 2.30)]. The resulting morphokinetic model was more accurate to predict live birth than conventional embryo morphology, as measured by AUC on an external dataset not used for model development [AUC 0.658 (95% CI 0.606 – 0.681) and AUC 0.575 (95% CI 0.537 - 0.614), respectively]. Regression analysis showed a significant difference between the three subgroups in which morphokinetic quality was classified as poor (C; subgroups AC, BC, and CC) compared to the reference group AA (adjusted $p < 0.001$). Moreover, odds ratio obtained in subgroups AC (good morphology/poor morphokinetics) and CA (poor morphology/good morphokinetics) indicated that poor morphokinetics is a better marker of low embryo competence than poor morphology (OR = 0.23; 95% CI 0.09-0.60 and OR=0.55; 95% CI 0.17-1.87, respectively).
Limitations, reasons for caution: We acknowledge that our study is limited by its retrospective nature and by the utilization of data generated in a single IVF center including only day 3 transfers.
Wider implications of the findings: Our results suggest that embryo morphokinetics is a more reliable predictor of live birth potential after day 3 fresh embryo transfers than embryo static morphology, thus providing valuable references to define embryo selection strategies in IVF practice.
Trial registration number: Not applicable

P-210 The impact of synchronicity of blastomere division during dynamic human embryo monitoring by a time-lapse on blastocyst development and quality.

N. Sayme[1], M. Kljajic[2], D.H.A. Maas[1], T. Krebs[1]

[1]Team Kinderwunsch Hannover, Dept. of Ob/Gyn, Hannover, Germany ;
[2]Universitätsklinikum des Saarlandes, Reproduktionsmedizin, Homburg, Germany

Study question: Does the synchronicity of blastomere division of embryo and type of media system have an impact on blastocyst development and quality.
Summary answer: The synchronicity of the cell cycle affects blastocyst development and blastocyst quality, while the media system only affects blastocyst quality.
What is known already: The serial image capturing with time-lapse devices is a noninvasive method that offers the possibility for 24-hour continuous monitoring of embryo development, increasing the quantity and quality of information without disturbing the culture conditions. Some of the most recent publications show correlations between cleavage times until 8-cell stage and blastocyst rate, expansion, and implantation. These published data suggest that morphokinetic observations can yield valuable information to aid the selection of embryos for transfer. The impact of single versus sequential culture media systems on time-lapse data also needs to be considered since the contradictory conclusions were reached recent studies.
Study design, size, duration: The synchrony of the second cell cycle (s2) was defined as the duration of the transition from a two-blastomere embryo to a four-blastomere embryo (s2 = t4–t3), while synchrony of the third cell cycle (s3) as the duration from a five-blastomere embryo to an eight-blastomere embryo (s3 = t8–t5). The injection time of ICSI was designated as "time zero" (t0), and computer software was used to calculate the time duration of the cycle.
Participants/materials, setting, methods: A total of 87 blastocysts from 20 patients undergoing the antagonist cycle for ICSI treatment between November 2019 and December 2019 were evaluated. All blastocysts were cultured in Embryoscope™ according to the manufacturer's specifications (Vitrolife, Sweden). The Gardner and Schoolcraft scoring system was used to describe blastocyst quality. Correlations between the data were calculated using logistic regression analysis. Statistical significance was defined as $p<0.05$. All statistical analyses were performed using SAS software.
Main results and the role of chance: Morphokinetic data showed that synchrony of the second cell cycle was significantly different (s2, $p<0,05$) between embryos which reached the blastocyst stage and those embryos that did not. Although, in contrast with what was previously published, the more careful analysis revealed that blastocyst quality was not affected by synchrony of the second cell cycle as morphocinetic parameter. On the other hand, synchrony of the third cell cycle did not significantly correlate with blastocyst formation potential, but this parameter showed a significant correlation with blastocyst quality (s3, $p<0,001$).

Besides Morphokinetic observations, the impact of single versus sequential culture media systems was investigated as well. For a single media system Gynemed 501CT (Lensahn, Germany) was used and for the sequential one Sydney IVF Cleavage Medium and Sydney IVF Blastocyst Medium (Cook Medical, Ireland) were used. Obtained results showed that there was not any difference between the synchrony of the second or third cell cycle regardless on the media system. What is more, blastocyst formation potential, blastocyst quality or pregnancy rate did not differ between media system groups.
Limitations, reasons for caution: The disadvantage of the relatively small patient cohort was balanced with a wider range of patients' age. Further research should link morphokinetic parameters with pregnancy rate and live birth rate as well.
Wider implications of the findings: The potential of the present findings might be helpful for a better understanding of the association between embryo morphokinetic parameters and blastocyst development. Furthermore, the culture media system result indicates that morphokinetic benchmarks can be developed and used irrespective of a laboratory's choice of culture media.
Trial registration number: not applicable

P-211 RNAseq reveals cumulus cell transcripts associated with developmental potential of bovine oocytes

Á. Martínez Moro[1,2], I. Lamas-Toranzo[2], E. O´Callaghan[3], A. Esteve-Codina[4], P. Lonergan[3], P. Bermejo-Álvarez[2]

[1]ProcreaTec, IVF Lab, Madrid, Spain ;
[2]INIA, Animal Reproduction Department, Madrid, Spain ;
[3]University College Dublin, School of Agriculture and Food Science-, Belfield-Dublin, Ireland ;
[4]Barcelona Institute of Science and Technology, Centre for Genomic Regulation, Barcelona, Spain

Study question: Does cumulus cell transcript abundance correlate with oocyte developmental potential in the bovine model?
Summary answer: The abundance of specific transcripts in cumulus cells from oocytes developing to blastocysts following IVF differs from those arresting before that stage.
What is known already: Cumulus cells serve essential functions during folliculogenesis, being connected to the oocyte through transzonal projections. Following in vitro maturation, cumulus cells are often removed and discarded, thereby constituting an interesting biological material on which to perform molecular analysis aimed to predict oocyte developmental potential. Previous studies performed to correlate the abundance of specific transcripts in cumulus cells with their developmental potential have yielded conflicting results. These studies have been based on candidate gene qPCR or microarray analyses. RNAseq provides significant advantages over these methods as it's more sensitive and specific and it is not limited to a subset of genes.
Study design, size, duration: Bovine cumulus cells were allocated into three groups according to the developmental potential of the oocyte: 1) oocytes developing to blastocysts following IVF (Bl+Cl+), 2) oocytes cleaving following IVF but arresting their development prior to the blastocyst stage (Bl-Cl+), and 3) oocytes not cleaving following IVF (Bl-Cl-). RNAseq was performed in 4 (Bl-Cl-) or 5 samples (Bl+Cl+ and Bl-Cl+) per group. Each sample contained cumulus cells from 10 cumulus-oocyte complexes (COCs).
Participants/materials, setting, methods: Bovine cumulus-oocyte complexes were obtained from slaughtered cattle and individually matured *in vitro* (IVM). Following IVM, cumulus cells were removed by hyaluronidase treatment, pelleted, snap frozen in liquid nitrogen and stored at -80 °C until analysis. Cumulus-free oocytes were fertilized and cultured *in vitro* individually and development was recorded for each oocyte. RNAseq was performed at Illumina platform with >30 M reads/sample. Differential expression was analysed by DESeq2 software.
Main results and the role of chance: Although the abundance of most transcripts detected in cumulus cells (19335) did not vary depending on the developmental potential of their corresponding oocytes, RNAseq revealed 1609, 1466 and 1420 differentially expressed genes (DEGs) for the comparisons Bl+Cl+ vs. Bl-Cl+, Bl+Cl+ vs. Bl-Cl- and Bl-Cl+ vs. Bl-Cl-, respectively, using a raw p value <0.05. These DEGs were narrowed down to 77, 80 and 32 DEGs for the comparisons Bl+Cl+ vs. Bl-Cl+, Bl+Cl+ vs. Bl-Cl- and Bl-Cl+ vs. Bl-Cl-, respectively, when an adjusted p value <0.05 was used. From these subsets of DEGs, 49, 50 and 18 DEGs, respectively, exhibited a fold change greater than 1.5. Focussing on DEGs in cumulus cells obtained from oocytes developing to blastocysts, 10 DEGs were common to both comparisons (10/49 from Bl+Cl+ vs. Bl-Cl+, 10/50 from Bl+Cl+ vs. Bl-Cl-). These DEGs correspond to 6 genes upregulated (HBE1, ITGA1, PAPPA, AKAP12, ITGA5 and SLC1A4), and 4 genes downregulated (GSTA1, PSMB8, FMOD and SFRP4) in Bl+Cl+ compared to the other groups.
Limitations, reasons for caution: Experiments were conducted in the bovine model. Although bovine folliculogenesis, monoovulatory ovulation and early embryo development exhibit considerable similarities with that of humans, caution should be taken when extrapolating these data to humans.
Wider implications of the findings: Selecting embryos with the highest developmental potential is essential in order to achieve successful outcomes following single-embryo transfer. Our findings suggest that the abundance of specific transcripts in cumulus cells could be used as a predictor of the developmental potential of their enclosed oocyte.
Trial registration number: not applicable

P-212 Epigenetic changes with maternal age in human eggs follow an inverse U-curve shape

T. Imbar[1], G. Karavani[2], P. Pash[3], N. Shecter-safrai[2], M. Safrai[2], T. Mordechai-daniel[2], D. Bauman[2], E. Reich[3], R. Rothman[3], T. Ajiro[3], M. Klutstein[3]

[1]Hadassah-Hebrew University Medical Center, Fertility preservation center- IVF unit, Jerusalem, Israel ;
[2]Hadassah-Hebrew University Medical Center, Fertility preservation center. IVF unit, Jerusalem, Israel ;
[3]Hebrew University, Institute of Dental Sciences, Jerusalem, Israel

Study question: How do epigenetic modifications change in human MI oocytes collected from media of unstimulated ovarian pieces with aging?
Summary answer: H3K9me2 repressive epigenetic mark in human oocytes may follow an inverse U-curve with age in a similar manner to the behavior of aneuploidies.
What is known already: Natural fertility in humans follows an inverse U-curve, where young females and women of advancing maternal age show reduced fertility rates. The mechanism or mechanisms that shape the fertility curve are unclear. A recent report shows that oocyte aneuploidies also follow such a U-curve, suggesting that chromosomal errors originating in oocytes determine the behavior of natural fertility in humans. Epigenetic changes are considered as one of "hallmarks of aging". These typically cause a decline of repressive marks in repressed loci and a contaminant repression of active genes. This phenomenon, previously uninvestigated in human eggs, is termed "epigenetic aging".
Study design, size, duration: After IRB approval, all participants, undergoing fertility preservation procedures, signed informed consent. From January 2019 until October 2019, we searched and collected oocytes from media remains after ovarian cortex pieces cryopreservation. Eight women included in the study. Age ranged from nine to 30 years. Two women received chemotherapy prior to fertility preservation procedure. We used a cohort of immature oocytes stained after retrieval from fertility age IVF patients as a control.
Participants/materials, setting, methods: Medium solution obtained from handling of ovarian cortical stripes, containing oocytes of different sizes and shapes together with cumulus cells. We separated different types of oocytes in the solution using a modified Andreasen pipette technique based on Stokes law of sedimentation. Oocytes found in the media were matured in vitro to MI and immuno-stained for the histone modification H3K9me2 .
Main results and the role of chance: Using our oocyte identification method we isolated oocytes that were not seen in the routine embryology lab medium search. After maturation in vitro we identified 3 GV , 23 MI and 6 MII oocytes. MI oocytes staining for epigenetic histone modification H3K9me2 revealed a low staining intensity in the younger patients (under 10 years) with a marked increase in the more mature, post-menarche (over 14 years), patients. Staining of MI oocytes from women 30 years and older showed and even lower intensity of this marker. Staining of the few GV oocytes we retrieved corroborated this staining pattern. Thus, the intensity of the H3K9me2 repressive epigenetic marker in human oocytes follows the shape of an inverse U-curve.
Limitations, reasons for caution: Staining results are from human oocytes during fertility preservation procedures, without gonadotropin induction. Moreover, the collection of oocytes was performed following a meticulous search and isolation of oocytes in the clinical lab. Thus, the epigenetic status may not reflect only their age, but also additional factors such as cellular stress.
Wider implications of the findings: Epigenetic changes involves in human oocyte maturation and may be part of the U-curve natural fertility seen in humans. Manipulating this effect, using epigenetic drugs, might enhance and improve our ability to preserve mature oocytes from young women with urgent need of fertility preservation
Trial registration number: not applicable

P-213 Big data is not always better – prediction of live birth using machine learning on time-lapse videos of human embryos

S. Hicks[1,2], T.B. Haugen[3], M. Iliceto[3,4], H.L. Hammer[1,2], J.M. Andersen[3], O. Witczak[3], M.A. Riegler[1], M.H. Stensen[4]

[1]Simula Metropolitan Center for Digital Engineering, Holistic Systems, Oslo, Norway ;
[2]OsloMet – Oslo Metropolitan University, Faculty of Technology- Art and Design, Oslo, Norway ;
[3]OsloMet – Oslo Metropolitan University, Faculty of Health Sciences, Oslo, Norway ;
[4]Fertilitetssenteret, IVF Laboratory, Oslo, Norway

Study question: Can interpretable machine learning (ML) methods be used on small amounts of data to predict human embryo viability?
Summary answer: Traditional supervised and unsupervised ML methods can predict human embryo viability using a small number of time-lapse videos.
What is known already: There is insufficient evidence to conclude that the introduction of time-lapse technology has improved live birth outcomes after ART compared with traditional embryo assessment. ML methods have shown to have much potential in analyzing images and may have predictive value in the assessment of embryos from time-lapse videos. All recent work uses some variation of deep neural networks that are known to require a lot of training data and are not easily interpretable. Thus, we propose to use interpretable and less data-demanding ML methods to predict embryo outcome.
Study design, size, duration: We used eight time-lapse videos of embryo development, up to day five. Two labels were assigned to the videos; positive for the embryos which resulted in a live birth and negative for the non-viable embryos. The dataset was evenly balanced with an equal number of positive and negative labels. 120 frames were extracted at evenly distributed intervals throughout the entire duration of each video in the dataset.
Participants/materials, setting, methods: Global image features (GF) were extracted from each video frame. GF have the advantage of being manually engineered, which are more interpretable when compared to features extracted through deep learning. We extracted 25 different features, where the best performing features were Tamura and AutoColorCorrelogram. Due to the small number of real time-lapse videos, Synthetic Minority Over-sampling Technique (SMOTE) was used to increase the sample size. Leave-one-embryo-out cross-validation was used to demonstrate generalizability and avoid overfitting.
Main results and the role of chance: The experiments can primarily be split into two distinct groups; supervised and unsupervised ML methods. The supervised experiments used the algorithms naïve Bayes and random forests and evaluated using leave-one-embryo-out cross-validation with and without SMOTE (1000% oversampling for both classes). The unsupervised experiments were done using simple K-means clustering (using two clusters) and X-means (number of clusters chosen automatically) with and without oversampling. The motivation behind choosing more traditional algorithms over modern ones, such as deep learning, was due to their efficiency in terms of training and inference time. Furthermore, the methods used are more interpretable and allow for a higher understanding of why and how the algorithms make a prediction. The supervised methods using SMOTE performed well and classified all eight videos correctly (sensitivity and specificity of 1). Without SMOTE, specificity and sensitivity fell between 0.375 up to 0.750 depending on which feature was used. The unsupervised methods also performed well. K-means with SMOTE correctly clustered all videos into the correct clusters (live birth and not live birth). Without SMOTE, one video was wrongly clustered. X-means automatically generated two clusters and classified all instances correctly using SMOTE. Without SMOTE, X-means was able to cluster about 60% correctly.
Limitations, reasons for caution: Since the size of the dataset was minimal, leave-one-embryo-out cross-validation and SMOTE were essential in the validation of this study. For future studies, larger datasets should be used with data collected from different sources to increase generalizability and the robustness of the validation.
Wider implications of the findings: Supervised ML methods have a potential for live birth prediction. Unsupervised learning may become an essential tool for the discovery of new embryo features for use in assessments. Our results suggest that Tamura features might be visual biomarkers for embryo video analysis.
Trial registration number: not applicable

P-214 Ooplasmic DNA Repair Mechanisms of the Human Gamete Appear to Be Linked to Oocyte Maturity

D. Keating[1], A. Parrella[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Does ooplasmic maturity affect the ability of MII oocytes to repair fragmented male genome DNA?
Summary answer: If the proportion of mature oocytes retrieved is not optimal, the oocytes are unable to overcome abnormal sperm chromatin fragmentation (SCF) and generate implantable embryos.
What is known already: It is known that following sperm penetration, the ooplasm replaces the protamines of the spermatozoon with histones and runs

through the male genome, repairing DNA for accuracy and eventual repair. This is particularly relevant in cases of high SCF, known to affect implantation. The achieved nuclear maturation by extrusion of the first polar body, not concurrently, is followed by ooplasmic readiness. We have shown previously that proportional nuclear maturity of the retrieved oocyte cohort has a crucial impact on ICSI outcome in terms of fertilization, embryo implantation, and live birth.
Study design, size, duration: This study included couples treated by ICSI using ejaculated spermatozoa between 2006 and 2019. Female partners were limited to ≤37 years of age to control for female-related gamete aneuploidy. Nuclear maturity of the retrieved oocyte cohort was assessed after the removal of cumulous cells. Cycles were allocated according to the proportion of nuclear maturity of the retrieved cohort: optimal (≥80%) and suboptimal (<80%).
Participants/materials, setting, methods: Ejaculate SCF was assessed by terminal deoxynucleotidyl dUTP transferase nick-end labeling (TUNEL). Five-hundred spermatozoa were assessed with a normal threshold of ≤15%. Female patients were stimulated for ovarian superovulation by pituitary suppression utilizing either gonadotropin-releasing hormone antagonist or agonist, were treated with daily gonadotropins, and were triggered with human chorionic gonadotropin when the lead follicle reached ≥17mm. Oocyte retrieval and ICSI were performed in the standard fashion. All cycles were performed at our center.
Main results and the role of chance: A total of 126 couples underwent 220 ICSI cycles that had an optimal oocyte maturity, and 119 couples underwent 202 cycles with suboptimal oocyte maturity. The average SCF for couples with normal and abnormal levels was 9.4±3% and 24.1±10%, respectively (P<0.0001). When oocyte maturity was optimal, couples with normal and abnormal SCF had comparable fertilization (74.0% vs. 70.8%), embryo implantation (22.2% vs. 22.3%), and clinical pregnancy rates (CPR; 32.8% vs. 37.9%).
For the suboptimal maturity group, couples with normal SCF had a fertilization rate of 72.4%, an embryo implantation rate of 20.2%, and a CPR of 30.5%. Couples with abnormal SCF had a comparable fertilization rate of 72.0%; however, the implantation rate was compromised (9.8%; P<0.05). Similarly, in these couples, CPR trended lower (30.5% vs. 17.8%).
To confirm the impact that proportional oocyte maturity has on the alleviation of male genomic defects, we assessed only cycles with abnormal SCF. In cycles where the proportion of MII oocytes was optimal, the fertilization rate was 70.8%, the implantation rate was 22.3%, and the CPR was 37.9%. Cycles with suboptimal maturity had a comparable fertilization rate (72.0%); however, embryo implantation (9.8%; P<0.05) and CPRs were impaired (17.8%; P<0.05).
Limitations, reasons for caution: While we limited female age to ≤37 years to control for female-related gamete aneuploidy, it is not possible to exclude all confounding factors with certainty. This study demonstrated that not only nuclear but also cytoplasmic maturity is required for a competent MII oocyte.
Wider implications of the findings: These findings confirm the existence of DNA repair mechanisms within the human oocyte and that this function is impaired in oocytes with ooplasmic dysmaturity. This was evident in the retrieved cohort with a suboptimal number of MII oocytes. Optimization of the superovulation protocol can overcome male genomic impairment.
Trial registration number: not applicable

P-215 The role of autophagy on embryonic development at blastocyst stage

N. Adel[1], H. Elmaghraby[2], D. Ghareeb[1], M. Elmahdy[2], M. Nabil[3]

[1]Alexandria university- Faculty of science, Biochemistry, Alexandria, Egypt ;
[2]Alexandria university- Faculty of medicine, Obstetrics and Gynecology, Alexandria, Egypt ;
[3]City of scientific researches and technological applications., Pharmaceutical and fermentation industries development center, Alexandria, Egypt

Study question: Does autophagy genes and proteins expression is deifferent between preimplantation embryos that developed to blastocyst and those which are arrested on day 5?
Summary answer: From all autophagy genes and proteins, Beclin 1 and LC3 expression is important for embryonic development to blastocyst on day 5.
What is known already: Autophagy is one of the molecular mechanisms that is important for the early embryonic development. It is a massive cytoplasmic degradation pathway mediated by the lysosome.
Autophagy provides a"recycling system", As it engulfs unnecessary macromolecules proteins and organelles, degraded them to amino acids afree fatty acids which are important for the neo-synthesis of another proteins important for embryo development, It was known that autophagy is highly activated immediately after fertilization, and also found that fertilized embryos lack autophagy can not implant.
Study design, size, duration: Our study is a prospective study, 60 embryos were derived from oocytes aspirated from twelve patients undergoing ICSI between September 2019 to November 2019 at Madina fertility center.Embryos were devided on day 5 in to two groups according to its developmental stage and its ability to form blastocyst:

Group A ----> Developed embryos (blastocyst formation) n=31.
Group B ----> Arrested embryos (No blastocyst formation) n=29.

Participants/materials, setting, methods: Oocytes aspirated from twelve patients were injected with sperm by conventional ICSI , the injected oocytes were incubated till day 5.
Embryos on day 5 were scored and devided in to two groups according to its developmental stage and its ability to form blastocyst:

Group A ----> Developed embryos (blastocyst formation)
Group B ----> Arrested embryos (No blastocyst formation)

then biopsied to evaluate expression of autophagic genes (ELISA) and proteins (RT-PCR) : LC3, PI3K, E2F, mTOR.
Main results and the role of chance: Developed embryos to blastocyst stage on day 5 (Group A) shows significantly higher LC3 relative gene expression (1.12±0.51), Beclin 1 relative gene expression (1.43±0.33) and Beclin protein expression (3.8±0.028) than their expression in embryos that failed to form blastocyst on day 5 (Group B) (0.72±0.17,P=0.03), (0.35±0.12,P=0.0001),and (3.14±0.05, P=0.0001), respectively.
While mTOR and PIK3C3 proteins expression were significantly higher in Group B (arrested embryos) than their expression within developed embryos (Group A) , P=0.007 and P= 0.0001 ,respectively.As well as the expression of E2F gene which is significantly lower within group A embryos (0.32±0.07) and remarkedly higher within group B embryos (4.38±1.16), P=0.0001.

	Developed (Group A)	Arrested (group B)	P
mTOR (ng)	1.13±0.053	1.23±0.09**	0.007
Beclin (ng)	3.8±0.028	3.14±0.05***	0.0001
PIK3C3 (ng)	18.6±1.4	24.5±1.5***	0.0001
LC3 relative gene expression fold	1.12±0.51	0.72±0.17*	0.03
Beclin 1 relative gene expression fold	1.43±0.33	0.35±0.12***	0.0001
E2F relative gene expression fold	0.32±0.07	4.38±1.16***	0.0001

Limitations, reasons for caution: larger sample size is needed to support our results.
Wider implications of the findings: Investigation of autophagy function (specially evaluation of expression of Beclin 1 and LC3 genes and proteins) in embryos at late developmental stage introduce a new diagnostic tools for embryos with impaired development and those with impaired implantation potential.
Trial registration number: 0303721

P-216 The influence of post-thaw culture period on the pregnancy outcomes of poor-quality blastocyst transfer

S.H. Shin[1], J.H. Cha[1], Y.J. Kim[1], S.K. Lee[1], J.H. Kim[1], D.H. Kim[1], H.Y. Kim[1], S.H. Back[1], M.J. Choo[1], J.H. Ahn[1], H.Y. Kim[1], K.A. Pak[1], J.S. Yoon[1], S.Y. Park[1]

[1]Agaon Fertility Clinic, IVF center, Seoul, Korea- South

Study question: Does post-thaw culture period affect the pregnancy outcomes of poor-quality blastocyst in single vitrified-thawed embryo transfer?

Summary answer: In poor-quality blastocyst transfer, short post-thaw culture period was higher the pregnancy outcomes compared to long post-thaw culture period.

What is known already: During vitrification and thawing, embryo can be damaged and stressed. Stressed embryo can be particularly sensitive to environmental influences during post-thaw culture. Therefore, it raised hypothesis that the post-thaw culture period may be affect the pregnancy outcomes.

Study design, size, duration: A retrospective cohort study of 428 vitrified-thawed single embryo transfer cycles was performed from January 2014 to October 2019. Cycles with surgical retrieved sperm, genetic diagnosis, oocyte donation, and advanced maternal age (≥38 years) were excluded. We compared rates of survival, biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and implantation according to post-thaw culture period.

Participants/materials, setting, methods: Cycles, that transferred single embryo of poor-quality, were divided into two groups according to post-thaw culture period; cultured overnight (21-23 h) in group A (n = 281) and short culture (3-5 h) in group B (n = 147). Blastocyst was graded according to the Gardner and Schoolcraft's classification system. Poor-quality was defined as excluding expansion grade 4-6 and ICM/trophectoderm grade A or B.

Main results and the role of chance: Cycle characteristics such as female age (33.4 ± 2.5 vs. 33.4 ± 2.4, p = 0.959), survival rate (96.6 % vs. 97.4 %, p = 0.651) were similar in the two groups. Rates of biochemical pregnancy (25.6 % vs. 44.9 %, p < 0.001), clinical pregnancy (18.1 % vs. 31.3 %, p = 0.002), and implantation (18.5 % vs. 32.0 %, p = 0.002) in Group B were higher than those of Group A. Group B tends to have a higher ongoing pregnancy rate (14.9 % vs. 22.4 %, p = 0.053) than Group A, but no significant difference.

Limitations, reasons for caution: This is a retrospective study. The quality at the time of blastocyst vitrification was not considered. Therefore, further studies are needed in order to support our results.

Wider implications of the findings: We analyzed poor-quality blastocyst, which can be assumed to be more stressed. This study suggests that poor-quality blastocyst may be sensitive to the in vitro environment. Therefore, the short post-thaw culture period of poor-quality blastocyst can improve the pregnancy outcomes.

Trial registration number: Not Applicable

P-217 The effect of inner cell mass and trophectoderm morphology on embryo ploidy, gender, and in vitro fertilization outcomes

C. Moutos[1], W. Kearns[2,3], S. Farmer[4], J. Richards[4], A. Saad[1], J.R. Crochet[1,4]

[1]University of Texas Medical Branch – Galveston, Obstetrics and Gynecology, Galveston, U.S.A. ;
[2]AdvaGenix, AdvaGenix, Rockville, U.S.A. ;
[3]Johns Hopkins University School of Medicine, Gynecology and Obstetrics, Baltimore, U.S.A. ;
[4]Center of Reproductive Medicine, Center of Reproductive Medicine, Houston, U.S.A.

Study question: Is there a relationship between inner cell mass (ICM) or trophectoderm (TE) grading and embryo gender, ploidy and *in vitro fertilization* (IVF) outcomes?

Summary answer: Higher ICM /TE grade embryos were more likely to be euploid and have better IVF outcomes. while no correlation exists between ICM/TE and embryo gender.

What is known already: Along with preimplantation genetic testing for aneuploidy (PGT-A), embryo morphology has been used in selecting which embryo to transfer during IVF/embryo transfer (ET) procedures. The relationship between embryo morphology and euploidy has been well established; the effects of ICM and TE grading separately on PGT-A and subsequent IVF outcomes remain uncertain.

Study design, size, duration: A retrospective chart review was conducted of patients age 21 to 47. Embryos that underwent PGT-A (N=3708) and subsequent elective single ET (N=539) from June 2007 to December 2018 were evaluated.

Participants/materials, setting, methods: Embryo data originated from patients receiving care at a single reproductive endocrinology practice. Primary outcomes of this study consisted of IVF outcomes among different embryo ICM and TE morphological grades. Secondary outcomes included embryo gender and ploidy. Statistical analyses were performed via Pearson's chi-squared tests.

Main results and the role of chance: ICM Grade A embryos (N=2214) were euploid in 57.1% of cases versus 45.5% and 31.2%, respectively, for ICM Grade B (N=1090) and Grade C (N=404), (P<0.0001). TE grading resulted similarly for euploidy (Grade A [N=1551] 62.0% vs. Grade B [N=1627] 48.3 % vs. Grade C [N=530] 26.2%, P<0.0001). Neither ICM (P=0.257) nor TE (P=0.079) correlated with gender.

In transferred, euploid embryos, the distribution included ICM Grade A (N=392), Grade B (N=116), Grade C (N=31) and TE Grade A (N=302), Grade B (N=209), Grade C (N=28).

Implantation rate (IR) (ICM Grade A 71.7% vs. Grade B 59.5% vs. Grade C 48.4%, P<0.0001), clinical pregnancy rate (CPR) (ICM Grade A 69.4% vs. Grade B 57.8% vs. Grade C 45.2%, P=0.0034), and ongoing pregnancy rate (OPR) (ICM Grade A 59.7% vs. Grade B 48.3% vs. Grade C 38.7%, P=0.013) are higher with better ICM grades.

TE grading resulted: IR (TE Grade A 73.8% vs. Grade B 63.2% vs. Grade C 35.7%, P<0.0001), CPR (TE Grade A 71.5% vs. Grade B 60.8% vs. Grade C 35.7%, P=0.0001), and OPR (TE Grade A 61.6% vs. Grade B 51.7% vs. Grade C 28.6%, P=0.0009). ICM and TE do not correlate with miscarriage, chemical, or ectopic pregnancy.

Limitations, reasons for caution: Limitations of this study include a patient population derived from a single facility. Additionally, the retrospective nature of this study leaves the possibility for introduction of unaccounted for confounding factors.

Wider implications of the findings: This large-scale study elaborates on the use of ICM and TE grading in predicting embryo gender, ploidy, and successful IVF outcomes. Our data suggest that both ICM and TE grading are important tools and should both be considered when selecting embryos for successful IVF outcomes.

Trial registration number: None

P-218 Human sperm selected by a direct swim-up without centrifugation is positively associated with high blastocyst formation rate in normal responders after intracytoplasmic sperm injection (ICSI)

J. Lee[1], S. Shim[1], M. Kim[1], S. Choi[1], I. Park[1], H. Sun[1], J. Kim[1]

[1]Liora Fertility Center, IVF center, Busan, Korea- South

Study question: Does human sperm selected by the direct swim-up (DU) without centrifugation affect development and quality grade of embryo and blastocyst formation rate after ICSI?

Summary answer: The human sperm selected by DU without centrifugation was significantly associated with high blastocyst formation rate, but unrelated to development and quality grade of embryo.

What is known already: Centrifugation could produce reactive oxygen species and impair sperm functions, and also induce sub-lethal damage. Therefore, we are still concerned about using centrifugation in the procedure of sperm preparation. Previous study suggested that the DU without centrifugation can minimize additional sperm preparation and should be used in assisted reproductive technology (ART) treatment as a reasonable alternative to improve the embryo development and clinical outcomes. However, there is insufficient evidence from previous studies to support this suggestion.

Study design, size, duration: A retrospective study of 76 (n = 1,365 retrieved oocytes) fresh ICSI cycles with normal responders was conducted from May 2018 to December 2019 at Liora Fertility Center. All cycles with severe oligoasthenoteratozoospermia, frozen sperm and surgically retrieved sperm were excluded. All women had undergone a GnRH antagonist protocol. Cycles were divided into two groups; DU without centrifugation (Group A, n = 49) and DU with centrifugation (Group B, n = 27).

Participants/materials, setting, methods: Sperm medium (1) was placed on the top of raw semen (1) in 5 tube. The supernatant with motile sperm was gently collected after the DU for 20 minutes, and then placed into 15 tube without the washing step using centrifugation. Then the 15 tube was stored in 6% CO_2 incubator at 35°C before ICSI. We compared the fertilization rate, embryo development, embryo quality grade and blastocyst formation rate between two groups.

Main results and the role of chance: There were no significant differences between Group A and B regarding mean female age (33.5 ± 3.9 vs. 34.6 ± 4.4, *p* = 0.257), mean male age (35.4 ± 4.5 vs. 37.1 ± 5.1, *p* = 0.137), mean number

of in *in-vitro* fertilization (IVF) attempts (0.3 ± 0.6 vs. 0.3 ± 0.8, $p = 0.864$), mean number of retrieved oocytes (18.8 ± 7.2 vs. 16.4 ± 7.2, $p = 0.156$), mean number of Metaphase II oocytes (13.2 ± 4.7 vs. 11.5 ± 5.0, $p = 0.140$), maturation rate (87.0% vs. 90.0%, $p = 0.272$), fertilization rate (76.4% vs. 75.4%, $p = 0.707$), arrested embryos rate (1.0% vs. 0.7%, $p = 0.309$). We also observed similar rates of high-quality 4-cell stage embryos on day 2 (22.1% vs. 26.9%, $p = 0.256$), high-quality cleavage stage (5- to 9-cell stages) embryos on day 3 (26.3% vs. 30.0%, $p = 0.434$), and high-quality blastocysts stage (≥BB grade, 32.8% vs. 33.5%, $p = 0.898$) in Group A and B, respectively. However, the Group A had significantly higher blastocyst formation rate (58.4% vs. 50.1%, $p = 0.036$) than Group B. Lastly, any microbial contamination during embryo culture was not discovered in two groups.

Limitations, reasons for caution: This retrospective study is based on a small sample size so that further study in a large sample is needed. We also need to investigate whether the human sperm selected by DU without centrifugation positively affect pregnancy rate in IVF cycles.

Wider implications of the findings: This method is possible to reduce the influence of impaired sperm motility and DNA damage by centrifugation. Additionally, it can help to reduce an embryologist workload as a simple sperm preparation and provide the cost effective in ART. We suggested that this method will improve safe and useful clinical application.

Trial registration number: not applicable

P-219 Can deep convolutional neural network (CNN) be used as a non-invasive method to replace Preimplantation Genetic Testing for Aneuploidy (PGT-A)?

A. Meyer[1], J. Dickinson[1], N. Kelly[1], H. Kandula[2], M. Kanakasabapathy[2], P. Thirumalaraju[2], C. Bormann[1], H. Shafiee[3]

[1]Massachusetts General Hospital, Obstetrics and Gynecology, Boston, U.S.A. ; [2]Brigham and Women's Hospital, Division of Engineering in Medicine- Department of Medicine, Boston, U.S.A. ; [3]Brigham and Women's Hospital, Department of Medicine- Harvard Medical School, Boston, U.S.A.

Study question: Can deep convolutional neural network (CNN) be used as a non-invasive method to accurately classify embryos based on their karyotype?

Summary answer: Trained CNN algorithm of cleavage and blastocyst-stage embryo images performed very well in identifying aneuploid embryos.

What is known already: Preimplantation genetic testing for aneuploidy (PGT-A) has become a widely utilized tool for screening and selecting embryos for transfer. There are limited studies showing the efficacy of this procedure in women under 35 years of age. Conversely, there are some reports demonstrating that younger patients have reduced cumulative pregnancy rates when utilizing PGT. These lower outcomes are likely due to inaccurate PGT-A results or due to the invasive procedures involved with embryo biopsy and cryopreservation. The goal of the study was to determine if deep-learning CNNs could be used as a non-invasive method to select chromosomally normal embryos for transfer.

Study design, size, duration: Using a retrospective dataset of embryos analyzed using a modified FAST-SeqS next generation sequencing method (Invitae, San Francisco, Ca) a deep neural network model was trained and tested using 3112 images to classify embryos as aneuploid or euploid. Embryo images captured on day 3 (D3) and day 5 (D5) were used to classify embryos based on karyotype.

Participants/materials, setting, methods: For D3 karyotype classification we developed a CNN with 2910 annotated images of cleavage embryos. We tested the performance using 202 D3 embryo images. For D5 karyotype classification we developed a CNN using 2906 images of annotated blastocysts. We tested the algorithm's performance using 201 blastocyst images.

Main results and the role of chance: The deep learning CNN trained to classify D3 embryos as aneuploid or euploid performed with a high specificity and thus was able to sufficiently identify 85.45% (CI: 77.46% to 91.45%) of aneuploid embryos. Similarly, the CNN used to classify embryos based on karyotype on D5 of development had a specificity of 75.23% (CI: 66.04% to 83%). These results objectively demonstrate that morphology-alone may be useful in identifying genetically abnormal embryos.

Limitations, reasons for caution: Images were obtained using a single imaging platform (EmbryoScope) at only 2 timepoints for karyotype classification.

Wider implications of the findings: These results demonstrate while networks can identify non-invasive markers for genetically-abnormal embryos, the need for markers to selecting genetically-normal embryos for transfer remains. This was the first report to our knowledge that's able to objectively determine without technician-bias or input that morphology-alone can be used to identify genetically abnormal embryos.

Trial registration number: not applicable

P-220 One step further: Prospective randomized controlled trial comparing one and two steps technique of embryo transfer.

P.E. Levi Setti[1], F. Cirillo[1], E. Morenghi[2], V. Immediata[1], V. Caccavari[3], A. Baggiani[1], E. Albani[1], P. Patrizio[4]

[1]Humanitas Research Hospital, Department og Gynecology- Division of Gynecology and Reproductive Medicine- Humanitas Fertility Center, Milano, Italy ; [2]Humanitas Research Hospital, Department of Biostatistics, Milano, Italy ; [3]Istituto Clinico Città Studi, Department of Gynecology- Division of Assisted Reproduction, Milano, Italy ; [4]Yale University- School of Medicine, Department of Obstetrics- Gynecology and Reproductive Sciences, New Haven, U.S.A.

Study question: To determine if there are differences in ease of use between two different embryo transfer's techniques: preload single step and post load double step approach.

Summary answer: Two step technique seems to reduce the difficult embryo transfer rate.

What is known already: Numerous published trials now document that the embryo transfer (ET) procedure has a huge impact on pregnancy and delivery rates after IVF. Difficult transfers should be avoided, as they are likely to reduce the chances of implantation and subsequent pregnancy rates. Free hand embryo transfers with soft catheters under ultrasound guidance is currently considered the best option in transferring embryos. However, in technically difficult ET the use of harder catheters is needed and this could impact in uterine contraction. Among soft catheters, it is not known which technique is preferable.

Study design, size, duration: prospective randomized unblinded controlled clinical trial, including 352 ecoguided ETs assigned to single step ET with Cook k-soft-5000 soft catheter or double step ET with Cook K-JETS-551910-S bulb tip catheter, from September 2017 to September 2019. The sample size was calculated on the basis of historical difficult ET rate encountered between 2014 and 2015. The randomization to one of the study branches was done by the operator just before the procedure, with a closed envelope.

Participants/materials, setting, methods: Inclusion criteria: 18 - 38 years old, BMI between 18 and 28, one thawed blastocyst transfers. Exclusion criteria: ICSI-TESE and pregestational test cycles. The primary outcome was difficult transfer rate, defined as presence of blood, required manipulation, need for instrumentation, stylet or tenaculum, multiple attempts, force and dilatation, for both groups. For the single step approach, the need for the outer sheath was considered as a difficult ET. The secondary outcome was clinical pregnancy rate.

Main results and the role of chance: A total of 352 ET's were performed. The two arms were homogeneous for age at ET and at freezing, endometrial preparation protocols, BMI, and duration of infertility. Among the entire population, 24.15% of the ET was defined as difficult. In the two branches, difficult transfer rate was significantly higher in the single step group than in the double step's one: 38.76% versus 9.20% respectively ($p<0.001$); the OR of difficult transfer with the double step was 0.16 (IC 95% 0.09 – 0.29). Clinical pregnancy rate was lower, however not significantly, in the single step group (42.13% versus 48.28%).

Limitations, reasons for caution: 18 different experienced operators participated in the trial, using a fixed distance transfer protocol. The differences in difficult transfer rate between operators were not analyzed. Conclusions about the pregnancy rate should not be generalized, since the sample analysis was not performed on this outcome.

Wider implications of the findings: Difficult transfer rate was significantly higher in the single step group. Further study about the correlation with ART outcomes are required.

Trial registration number: NCT03161119

P-221 Euploidy rates between either oral dydrogesterone (DYG) primed ovarian stimulation protocol or the GnRH antagonist (GnRH-ant) protocol in 780 patients in the first PGT-A cycle

L. Yang[1], K. Luo[1], G. Lu[1,2,3], G. Lin[1,2,3], F. Gong[1,3]

[1]Reproductive and Genetic Hospital of CITIC-XIANGYA, Reproductive and Genetic Hospital of CITIC-XIANGYA, Changsha, China ;
[2]Key Laboratory of Reproductive and Stem Cell Engineering- National Health and Family Planning Commission, Key Laboratory of Reproductive and Stem Cell Engineering, Changsha, China ;
[3]Institute of Reproducitve and Stem Cell Engineering- Basic Medicine College-Central South University, Institute of Reproducitve and Stem Cell Engineering-, Changsha, China

Study question: whether differences in euploidy rates between oral dydrogesterone (DYG) primed ovarian stimulation protocol and the GnRH-ant protocol in the first PGT-A cycle.
Summary answer: DYG is comparable to GnRH-ant protocol in terms of euploidy rates at OPU in the first PGT-A cycle.
What is known already: It is well known that successful meiosis in the IVF laboratory is inversely correlated with maternal age. However, it is unclear whether other factors, particularly the IVF process, can interfere with normal oocyte physiology. Oral treatment with DYG inhibits the pituitary LH surge during ovarian stimulation. Previous reports indicated that both the number of MII retrieved and pregnancy rates from these oocytes are comparable to short protocol of GnRH agonists during IVF cycles. It is worth investigating whether ovarian stimulation protocol in the first PGT-A cycle affects euploidy status in DYG protocol and the GnRH-ant protocol.
Study design, size, duration: This single-center, retrospective cohort study included patient who underwent the dydrogesterone (DYG, study group) or the GnRH-ant protocol (control group) in the first PGT-A cycle from November 2017 to May 2019 in Reproductive and Genetic Hospital of CITIC-XIANGYA. The study group and control group both included 390 cycles, obtained from 408 and 534 cycles respectively by propensity matching.
Participants/materials, setting, methods: We retrospectively evaluated in the first PGT-A cycle patients who underwent the dydrogesterone (DYG, study group) or the GnRH-ant protocol (control group). Propensity matching was used to identify a propensity matched control group from a cohort of 534 cycles, based on age, BMI, and AMH with a 1:1 ratio. Patient's demographic, baseline, and cycle characteristics were obtained for each cycle from our electronic medical record. The main outcome was euploidy rate.
Main results and the role of chance: 780 cycles were included in the study. The study group and control group both included 390 cycles. There was no difference in patient baseline and cycle characteristics between the two groups. The number of oocytes retrieved (6.08±5.02 vs. 6.41±4.68, P =0.34), MII (4.97±4.09 vs. 5.15±3.78, P = 0.42), embryos biopsied (1.88±2.57 vs. 1.79±2.01, P = 0.59), and embryos testing (1.88±2.57 vs. 1.79±2.01, P = 0.59) were no statistic difference between study and control groups. Due to some cycles did not form biopsy blastocysts. Therefore, cycles using NGS testing were 262 in study group and 263 in the control group respectively. The main outcome was that the euploid rate per embryo biopsied was not significantly different between study and control groups (DYG vs. GnRH-ant: 34.9%±2.4% vs.40.2%±3.97%, P = 0.116).
Limitations, reasons for caution: It is a retrospective study and subject to bias from confounding factors.
Wider implications of the findings: DYG and GnRH-ant protocol result in comparable euploid rates. Therefore, ovarian stimulation protocol with DYG should be considered a valid option in freeze-all PGT-A cycles, in view of its demonstrated effectiveness and known safety enhancement.
Trial registration number: not applicable

P-222 Prediction of blastocyst formation based on very early-cleavage embryos parameters by time-lapse monitoring

H. De Martin[1,2], G. Alecsandra[1], M. Fujii[1], M. Conatti[1], M. Nakano[2], T. Bonetti[3,4], P. Monteleone[2,5]

[1]Monteleone - Human Reproduction Center, Embriology, Sao Paulo / SP, Brazil ;
[2]Universidade de São Paulo USP, Gynecology Discipline - Obstetrics and Gynecology Department, São Paulo, Brazil ;
[3]Monteleone - Human Reproduction Center, Scientific Department, Sao Paulo / SP, Brazil ;
[4]Universidade Federal de São Paulo, Gynecology Department, São Paulo, Brazil ;
[5]Monteleone - Human Reproduction Center, Clinical Director, Sao Paulo / SP, Brazil

Study question: Is very early-cleavage embryos parameters, as soon as the second cell cycle (CC2), based on time-lapse monitoring (TLM) useful to predict blastocyst formation?
Summary answer: The time to embryos progress from two to three-cell (CC2) predict blastocyst formation. Optimal CC2 time-range is 8 to 13 hours, leading to 78.2% blastocysts
What is known already: Assisted reproductive technologies success is dependent of a number of factors, but embryo selection is a crucial step. The cell number during cleavage has been described as a key factor for selecting embryos, as slow or fast cleavage embryos shows reduced implantation potential. The introduction of TLM allowed additional noninvasive criteria to best embryo selection and markers of embryonic kinetic from the first-cleavage onwards has been studied and applied in algorithms for best embryo choice. Although early cleavage parameters may be predictive of blastocyst development and even implantation, this approach has gathered little attention and a consensus is not stablished.
Study design, size, duration: Retrospective cohort study used prospectively collected data from 671 ICSI cycles from 486 patients (37.7±4.2 years) between April/2018 and September/2019 in a private reproductive medicine center. A total of 4724 mature (MII) oocytes were fertilized by ICSI and cultured in a time-lapse monitoring system (Embryoscope®). Embryos were recorded since fertilization until the blastocyst formation. The time for pronuclei fading (tPNf), two-cell (t2), three-cell (t3), four-cell (t4), five-cell (t5), eight-cell (t8), nine-cell (t9) were evaluated.
Participants/materials, setting, methods: The early-cleavage characteristics were analyzed and correlated to blastocyst formation. The periods between t2-t3 (cell cycle two – CC2), between t3-t5 (CC3) and between t5-t9 (CC4) were calculated. The blastocysts were also classified according to morphology by Gardner's classification and those with trophoectoderm grade ≥3 and inner cell mass A or B were considered top-quality. The blastocyst rate (BlastR) was calculated by the number of blastocysts formed per number of 2PN in each category.
Main results and the role of chance: From the 4724 MII oocytes injected, 3622 were normally fertilized (2PN). From those, 3552 cleaved into two-cells (t2=28.5±6.7 hours), 3500 attained three-cell (t3=38.6±7.3 hours), 3399 got five-cell (t5=51.0±10.0 hours) and 3077 attained nine-cell (t9=73.7±12.2 hours). The mean time of CC2 was 10.2±4.7, CC3 was 12.6±6.7 and CC4 was 23.3±9.1 hours. A total of 2341 embryos developed to blastocyst stage (64.6%) on days 5 (n=2108, 90.0%) or 6 (n=233, 10.0%). We evaluated the blastocyst rate (BlastR) according to CC2 varying from zero (for embryos who had tripolar mitosis and did not had two cells observed) to ≥20 hours. For CC2<1.0 hour the mean BlastR was 20.4%, for CC2 between 1 and 7 hours the mean BlastR was 46.2%. The highest BlastR (78.2%) occurred for embryos presenting CC2 between 8 and 13 hours. After that, the BlastR progressively decreased for embryos with CC2≥14 hours (38.9%), attaining a mean of 12.2% for CC2≥20 hours. Among the blastocysts developed, we assessed the top-quality blastocyst rate (TQ-BlastR) and the same pattern was observed: CC2<1.0 hour: TQ-BlastR=17.0%; CC2 between 1 and 7 hours: TQ-BlastR=20.0%; CC2 between 8 and 13 hours: TQ-BlastR=55.5%; CC2≥14 hours: 42.7%. There was no TQ-Blast formed when the CC2 was ≥18 hours.
Limitations, reasons for caution: This is a retrospective study evaluating the association of CC2 and blastocyst formation. The choice of blastocysts for transfer was based on blastocyst morphology on day 5 or 6 and did not take into account the CC2. Then, we could not evaluate the impact of CC2 on pregnancy success.
Wider implications of the findings: Based on our findings, we can underline that time of CC2 predict blastocyst formation and can be used for selecting day-3 embryos for transfer and contribute to embryo development prognostic. The CC2 is an auspicious candidate to be included in the time-lapse algorithm and should be further validated for that.
Trial registration number: not applicable

P-223 Comparison between two oil layer thickness covering on human embryo culture: a prospective study

H. Bahri[1], M. Benkhalifa[2], W. Zidi[3], Z. Mhamdi[4], H. Garai[4], O. Triki[4], K. Ben Rhouma[4], G. Merdassi[4]

[1]Alyssa Fertility Group, Alyssa Clinic / HB laboratory, tunis, Tunisia ;

[2]Alyssa Fertility Group / Faculty of sciences of Bizert, Alyssa Clinic / Biochemistry department of La Rabta Hospital, tunis, Tunisia ;
[3]Faculty of sciences of Bizert, Biochemistry department of La Rabta Hospital, tunis, Tunisia ;
[4]Alyssa Fertility Group, Alyssa Clinic, tunis, Tunisia

Study question: Does the light paraffin oil layer thickness used for in vitro embryo culture improve in vitro development of human embryos and pregnancy rate?
Summary answer: The success rate of embryo implantation and biochemical pregnancy (BP) was significantly higher with *in vitro* embryo culture (EC) using a thicker cover of light-paraffin-oil.
What is known already: The oil overlay in embryo culture systems prevents medium evaporation helps to maintain appropriate pH and osmotic conditions and protects from volatile organic compounds and microbial contamination. In classic procedures, once microinjected, the oocytes are transferred into a culture dish of separated embryo culture medium drops (1 – 3 oocytes per drop of 25µl) covered with approximately 1 mm of paraffin oil (from the top of the drops). The risk of the infiltration of contaminating agent becomes higher after 3 days of culture.
Study design, size, duration: 168 patients (20-40years) undergoing in-vitro fertilization (IVF) treatment by intracytoplasmic-sperm-injection (ICSI) were enrolled in our prospective study between October 14 – December 23, 2019. After ICSI, 833 oocytes were allocated into 2 groups according to the thickness of oil covering the embryo culture medium (ECM): 1mm or 4mm. Statistical-analyses by SPSS 20.0 (SPSS Inc., Chicago-IL-USA) with Kolmogorov-Smirnov test for normality, Student-t test for parametric comparisons and Mann-Whitney test for non-parametric comparisons with $p<0.05$ as statistically-significant.
Participants/materials, setting, methods: Group-1: Protected culture (PC+): 520 matured oocytes with EC in dishes containing single layer of 400µl ECM covered by ~200µl of light-paraffin-oil (Ovoil, Vitrolife-Sweden) (~4mm from the ECM surface). Group-2 (PC-) 313 matured oocytes with EC in dishes covered by light-paraffin-oil (1mm of thickness from the top of the drops). Sage1-step (Origio-Denmark) was used as ECM. EC was into trigaz incubators. BP was defined as a positive βHCG-test after 2 weeks from the embryo transfer.
Main results and the role of chance: Embryo morphology was recorded on days 3 and 5. A total of 139 PC+ and 97 PC- day3 embryos and 59 PC+ and 46 PC- day5 embryos were analyzed.
There were no differences between the two groups in terms of mean of age, number of collected oocytes, and number of matured oocytes, cleavage rate, top quality day3 embryo rate and the number of transferred embryos. The proportion of top quality day3 embryos was 66.66% [0% – 100%] vs 75.00% [0% – 100%] in PC+ and PC- groups respectively. However, Blastulation rate was significantly higher in PC+ group than in PC- with 61.55% ±2.77% vs 57.73% ±2.54%; (p=0.001) respectively. Pregnancy rate per transfer was evaluated in 69% of the total patients. When the unknown βHCG test results were eliminated, the biochemical pregnancy rate was higher in the PC+ group compared to PC- group with 41.2% vs 35.4%; (p< 0.001) respectively.
Limitations, reasons for caution: The design in this study is not a randomized controlled trial, although it is prospective. Moreover, different origin of sperm (Ejaculated and testicular) were used to fertilize corresponding oocytes.
Wider implications of the findings: These results should interest embryologists who want to improve embryo culture conditions. More studies must be done with a larger cohort of oocytes.
Trial registration number: Not applicable

P-224 Reducing oxygen concentration from 5% to 3% after embryo compaction decreases the aneuploidy rate of the derived blastocysts. Analysis of 119 sibling zygotes

A. Ruberti[1], M.G. Minasi[1], E. Cursio[1], A. Colasante[1], S. Muzzì[1], G. Pirastu[1], D. Paccagnini[1], S. Gatti[1], A. Caragia[1], P. Greco[1], E. Iovine[1], A. Greco[1], M.T. Varricchio[1], E. Greco[1]

[1]European Hospital, Medicina della Riproduzione, Rome, Italy

Study question: Does a reduction in incubation oxygen concentration from 5% to 3%, from day-3 of culture in advance, affect blastocyst's quality?
Summary answer: Reducing oxygen concentration from day-3 of culture allows to obtain a higher blastocyst number available for the transfer compared to fixed 5% oxygen concentration.
What is known already: It is well established that culturing embryos in an oxygen concentration lower than the atmospheric one will increase clinical and live birth rates. The oxygen tension most widely employed is 5%. Based on the observation that the levels of oxygen in the uterus are lower than in the oviduct, it has been postulated that a further reduction in oxygen tension after embryo compaction could more closely mimics the natural environment, better supporting the embryo's physiology. Few studies comparing different oxygen values have been published with conflicting results and the optimal range of oxygen concentration has still to be determined.
Study design, size, duration: From September to December 2018, 12 patients undergoing to preimplantation genetic testing for aneuploidy cycles were enrolled in the study. The mean female age was 36.7±4.1 years old. Inclusion criteria were at least 6 normal fertilized oocytes. Exclusion criteria were: genetic diseases, severe male factors and egg donation cycles.
Participants/materials, setting, methods: Sibling zygotes were divided in 1:1 ratio in two groups. All cultures were performed in sequential media with a change-over on day-3. In the standard oxygen (SO) group, zygotes were maintained at 5% of oxygen tension for the whole culture. In the low oxygen (LO) group, zygotes were maintained at 5% until day-3 of culture and then moved to 3% of oxygen tension for the remaining days.
Main results and the role of chance: A total of 119 normal fertilized zygotes were obtained, 62 and 57 of them were cultured in SO and LO groups, respectively. The good/excellent day-3 embryo quality was comparable in the two groups: 80.6% (N=50) and 87.7% (N=50) in SO and LO groups, respectively (NS). The blastocyst formation rate was similar in the two groups: 56.5% (N=35) and 52.6% (N=30) in SO and LO groups, respectively (NS). In SO group, 15 blastocysts were obtained on day-5, 15 on day-6 and 5 on day-7. In LO group, 14 blastocysts were obtained on day-5, 16 on day-6 and none on day-7. A total of 30 and 27 blastocysts were biopsied in SO and LO groups, respectively. The percentage of blastocyst available for the transfer was statistically higher in LO group (81.5%, N=22) compared to SO group (46.7%, N=14; p=0.0124). In 3 and 1 blastocysts it was not possible to obtain the genetic result, in the SO and LO groups, respectively.
Limitations, reasons for caution: This is a pilot study enrolling few patients with a high oocyte's number. Data on clinical outcomes, such as implantation, clinical pregnancy, live birth and miscarriage rates as well as follow-up data on babies born, are still missing. These preliminary results need to be confirmed on an increased sample size.
Wider implications of the findings: It has been reported that euploidy rate can greatly vary among different infertility centers demonstrating that chromosomal abnormalities can be partially iatrogenic. To set-up the optimal culture conditions in order to obtain the highest number of transferable blastocysts could enhance the efficiency of infertility treatments.
Trial registration number: not applicable

P-225 The effect of women's age on timing, morphology and implantation of the competent blastocyst – a multicenter cohort study

M. Buhl Borgstrøm[1], M.L. Grøndahl[1], T. Wirenfeldt Klausen[2], A. Kjærgard Danielsen[3], T. Thomsen[4], U. Schiøler Kesmodel[5], I.T.C.B.P.G. Inclusive: The Competent Blastocyst Project Group[1]

[1]Herlev University Hospital, The fertility clinic, Herlev, Denmark ;
[2]Herlev University Hospital, Department of hematology, Herlev, Denmark ;
[3]Herlev University Hospital, Department of gastroenterology, Herlev, Denmark ;
[4]Herlev University Hospital, Department of Anaesthesiology, Herlev, Denmark ;
[5]Aalborg University Hospital, Department og Obstetrics and Gynecology, Aalborg, Denmark

Study question: Is the age of women undergoing assisted reproductive technology (ART) associated with timing, morphology and implantation of the competent blastocyst?
Summary answer: The effect of age on timing and morphology was modest, while initial hCG rise was lower for the youngest women.
What is known already: Within ART, the age of a woman is the most prognostic parameter for her chance to conceive. Both women's age and ploidy fail to be reflected in routine embryo assessment. We have previously shown that initial hCG rise in singleton pregnancies after single cleavage stage embryo transfer is higher with increasing age in both in vitro fertilization (IVF) and

intracytoplasmic sperm injection (ICSI) suggesting that age may influence trophoblast differentiation and placentation. Hence, the question remains whether the competent blastocyst develops in the same way regardless of women's age or if age imposes an alteration in timing, morphology and implantation?

Study design, size, duration: This is a multicenter historical cohort study based on exposure and outcome data from 7246 women, who between 2014 and 2018 underwent controlled ovarian stimulation (COS) or Frozen Embryo Transfer (FET) followed by single blastocyst transfer and hCG measurement, resulting in singleton pregnancy. These data were linked to the Danish Medical Birth Registry using the women's unique personal identification number (CPR). Resulting in a total of 5466 women with a live birth being included.

Participants/materials, setting, methods: Exposure (age) and outcome (Gardner's score (1-6), inner cell mass (ICM)(A,B,C), trophectoderm (TE) (A,B,C) and hCG) data were collected from the fertility database, Danish Medical Data Center, used by the 16 participating private and public fertility clinics. All COS cycles (IVF and ICSI) and FET cycles (natural and substituted), were included. Exclusion criteria were cycles with pre-implantation genetic testing and donated oocytes. The analysis was adjusted for male age, female smoking, female BMI, diagnosis and clinic.

Main results and the role of chance: The women's mean age for COS/FET was 32.4/32.6 years. Adjusted analysis of age and stage in COS treatments showed that for every one-year increase in age there was a 5% reduced probability of the competent blastocyst assessed as being in a high stage at transfer (OR 0.95, 95% CI (0.93;0.98)). Adjusted analysis of age and TE (A,B,C; A being the highest category) in FET treatments showed that for every one-year there was a 3% increased probability of the competent blastocyst assessed as being in a high TE category. Other comparisons related to the effect of age on timing and morphology did not reach statistical significance.

HCG values followed an identical pattern in COS and FET, with the lowest hCG values in the youngest age group for both treatments, 95.3 (IU/L) for COS and 130.9 (IU/L) for FET. Comparison between hCG values in women 18-24 years and 25-29 years in those receiving respectively COS and FET treatment, showed a significantly lower levels in the youngest women, with a significant mean difference in the adjusted analyses COS: -20.9, 95% CI (-35.7;-6.1)); FET: -21.8, 95% CI (-42.1;-1.5). Other comparisons did not reach statistical significance.

Limitations, reasons for caution: The blastocyst morphology was subjectively assessed and inter observer differences may have influenced the results. However, adjusting for clinic takes the potential inter clinic variation in embryo scoring into consideration.

Wider implications of the findings: Our study supports an association between young age and lower initial hCG-values after ART-treatment irrespective of mode of treatment, which should be born in mind when evaluating hCG-measurements.

Whether these findings correlate to the embryo or the endometrium need further clarification.

Trial registration number: not relevant

P-226 Paternal influence on mitochondrial DNA levels in day 3 euploid embryos: a retrospective pilot study.

Y. Mourad[1], M. Bazzi[1], V. Massaad[2], C. Afeich[2], G. Raad[1], J. Mortada[3], F. Fakih[4], R. Yazbeck[5], M. Chartouny[5], R. Zahwe[1], C. Fakih[1]

[1]Al Hadi Laboratory and Medical Center, Ivf, Beirut, Lebanon ;
[2]Lebanese University, Master in Reproduction and Development, Fanar, Lebanon ;
[3]Al Hadi Laboratory and Medical Center, Molecular Biology, Beirut, Lebanon ;
[4]Al Hadi aboratory and Medical Center, Ivf, Beirut, Lebanon ;
[5]Lebanese University, Faculty of Medicine, Hadath, Lebanon

Study question: Is there any paternal influence on the mitochondrial DNA levels in day 3 euploid embryos?

Summary answer: Higher mitochondrial DNA (mtDNA) levels were detected in day 3 euploid embryos of non-normozoospermic men compared to normozoospermic.

What is known already: Early paternal influence on pre-implantation embryo development was considered to be related to sperm cytoplasmic factors such as centrioles. Recently, double-stranded sperm DNA damage was shown to be associated with a delay in cleavage embryo kinetics. Furthermore, altered cleavage divisions may lead to an abnormal distribution of mitochondria between blastomeres. In parallel, oocyte may have the capacity to modulate and repair, to certain extent, the sperm genome. In this study, we hypothesize that poor sperm quality may modify the oocyte's mtDNA levels as a part of the repair mechanism.

Study design, size, duration: A retrospective pilot study was conducted at AL Hadi Laboratory and Medical Center, between January 2019 and January 2020.The study included the data analysis of 170 embryos obtained from 28 infertile couples undergoing pre-implantation genetic screening (PGS) for aneuploidy. Couples were categorized based on sperm indices into: normozoospermic (n= 13) and non-normozoospermic (n= 15) groups. Non-normozoospermia was defined by the presence of at least one abnormal sperm parameter according to the World Health Organization 2010.

Participants/materials, setting, methods: PGS data collected from couples with maternal age <38 years, a history of unsuccessful IVF treatments, and/or previous spontaneous miscarriages were included. Evaluation of embryo quality was carried out in accordance with Alpha-ESHRE consensus. Fresh embryos derived from fresh gametes were biopsied on day 3. Genomic and mitochondrial DNA levels were quantified using next generation sequencing (Ion Torrent, ThermoFisher Scientific, Lebanon). Implantation rates per transferred fresh embryo were analyzed.

Main results and the role of chance: From the total of 170 assessed embryos, 73 were identified as euploid. No statistical significant trend was observed in the percentage of euploid embryos between the normozoospermic and non-normozoospermic groups (49% and 38% respectively). In contrast, a higher mtDNA levels were detected in the non- normozoospermic group compared to the normozoospermic ($p = 0.0232$). Implantation and fertilization rates showed no significant difference between the two comparable groups ($p > 0.05$).

Limitations, reasons for caution: This study provided a possible effect caused by paternal factors on embryonic mtDNA levels. However, to determine the true extent of this clinical outcome, a prospective design with a larger sample size is necessary to corroborate the current findings.

Wider implications of the findings: The biological mechanism(s) underlying the association between paternal factors, oocyte repair system, and mitochondrial DNA are unknown. Hence, this topic is of considerable importance in future assessment of the role of mitochondria in oocyte ability to repair any sperm defect that could alter embryo development.

Trial registration number: Not applicable

P-227 Clinical factors affecting meiotic spindle imaging of oocytes by PolScope before ICSI

S.J. Kim[1], J. Jeong[1], S. Kim[1], T.H. Kim[1], J.H. Eum[1], W.S. Lee[1], S.W. Lyu[1]

[1]Fertility Center of CHA Gangnam Medical Center- CHA University School of Medicine, Department of Obstetrics and Gynecology, Seoul, Korea- South

Study question: Are there clinical factors associated with the visibility of meiotic spindle of oocytes when performing intracytoplasmic sperm injection (ICSI)?

Summary answer: Longer gonadotropin stimulation was associated with oocytes of no visible spindle, but not the ovarian reserve and female age.

What is known already: In the laboratory, Polarized light microscopy (PolScope) has been used to locate the meiotic spindle and inject oocytes without damaging spindle. Meiotic spindle imaging by PolScope has been studied to predict oocyte quality. Moreover, some studies analyzed various environmental conditions affecting meiotic spindle. However, there are few papers on the relationship between clinical factors and oocyte meiotic spindle.

Study design, size, duration: This retrospective study was conducted at a single fertility center. A total of 717 mature oocytes of 140 patients were selected for spindle analysis. Fertilization and embryo development of each oocyte were analyzed with respect to the observed spindle pattern. We evaluated multiple clinical factors as a predictor of oocytes with no visible spindle.

Participants/materials, setting, methods: From October 2017 to April 2019, patients who underwent ICSI in previous in vitro fertilization treatment but have failed pregnancy more than 2 times were recruited. The meiotic spindle angle of oocytes was defined as the angle of spindle deviation to the first polar body and graded as the follows: a, 0-5°; b, 6°-15°; c, 16°-30°; d, 31°-45°; e, >46°; non-visible spindle. Statistical analysis was carried out using the Chi-square test and multivariate regression analysis.

Main results and the role of chance: The mean age, anti-Mullerian hormone (AMH), and duration of stimulation were 39.6 ± 3.6 years, 2.25 ± 3.04 ng/ml,

and 9.0 ± 2.5 days, respectively. The number of oocytes retrieved and the number of mature oocytes retrieved were 7.8 ± 6.6 and 5.3 ± 4.8, respectively. The distribution of the oocytes according to grade were as follows: a (32.6%), b (31.7%), c (18.0%), d (7.1%), e (6.0%), and non-visible (4.6%). Fertilization rates were similar except non-visible grade: a (73.9%), b (74.0%), c (72.9%), d (72.5%), e (76.7%), and non-visible (51.5%). However, the cleavage rate and good quality of cleavage embryo formation rate were not different among the grades. When evaluating for factors predicting oocyte of non-visible spindle, the duration of gonadotropin stimulation was the only relevant factor (adjusted Odds Ratio (aOR) 1.218, 95% CI (1.005-1.477)). However, Female age (aOR 1.064 (0.929-1.220)), body mass index (aOR 1.092 (0.901-1.323)), AMH (aOR 0.976 (0.806-1.182)), and the number of oocytes retrieved (aOR 1.124 (0.948-1.333) were not associated with spindle nondetection.

Limitations, reasons for caution: The retrospective study design and the small number of study subjects are limitations. We only focused on clinical factors; therefore, further studies are needed to evaluate the laboratory factors such as temperature and pH.

Wider implications of the findings: Oocytes with non-visible spindle showed significantly lower fertilization rates. Meiotic spindle angle does not affect the oocyte quality represented by fertilization rate, cleavage rate, and good quality of cleavage embryo formation rate. The duration of gonadotropin stimulation was the only clinical factor related to oocytes of no visible spindle.

Trial registration number: Not Applicable

P-228 Laser assisted hatching at cleavage stage before the subsequent trophectoderm biopsy: to perform or not to perform?

M.G. Minasi[1], E. Cursio[1], A. Colasante[1], S. Muzzì[1], G. Pirastu[1], D. Paccagnini[1], S. Gatti[1], C. Arrivi[1], A. Greco[1], P. Greco[1], M.C. Greco[1], M.T. Varricchio[1], A. Ruberti[1], E. Greco[1]

[1]European Hospital, Center for Reproductive Medicine, Rome, Italy

Study question: Is the laser assisted hatching (LAH) day-3 zona pellucida (ZP) opening, performed in order to facilitate the subsequent biopsy at blastocyst level, safe?

Summary answer: To perform LAH at cleavage stage is safe and does not impair the ploidy nor the implantation rate in the deriving blastocysts

What is known already: Preimplantation genetic testing (PGT) is widespread used to select the embryo with the highest implantation potential. Currently, the blastocyst is the preferred developmental stage to be biopsied. A strategy is open the ZP with laser at embryo level in order to favorite the subsequent blastocyst hatching, simplifying the biopsy procedure. This method, however, has been suggested to negatively interfere with embryo development. Another possibility, hypothesized to be safer, consist of let the embryos undisturbed during the whole culture performing the ZP opening directly during the biopsy. A complete evaluation of the risks associated with these methods is still missing.

Study design, size, duration: Retrospective analysis of 491 PGT cycles. According to our laboratory procedure, when a cycle was allocated in sequential culture media all the developing day-3 embryos underwent to LAH during the media change-over (LAH group). When single-step media was employed, all embryos were maintained undisturbed in culture and LAH was performed directly during the biopsy at blastocyst stage (NO-LAH group). The allocation in the two culture media is randomly based on weekly rotation

Participants/materials, setting, methods: All cycles were performed from June 2015 to December 2019: 393 were allocated in LAH and 98 in NO-LAH groups, respectively. All culture were carried out in the same time-lapse incubator at 37°C, 5%O_2 and 6%CO_2. Mean female ages were 38.8±4.1 and 37.4±3.9 year old in LAH and NO-LAH groups, respectively (p=0.002). Monogenic diseases, structural rearrangements and egg donation cycles were excluded from the analysis. All embryo transfers were performed on a subsequent cryopreserved cycle

Main results and the role of chance: In LAH group, 3706 oocytes were collected, 2747 of them were mature and were injected. In NO-LAH group 720 out of 1013 retrieved oocytes were mature and injected. The fertilization rates were 76.2% (N=2093) and 80.0% (N=576) in LAH and NO-LAH groups, respectively (p=0.0324). A total of 2081 and 576 day-3 embryos were obtained in LAH and NO-LAH groups, respectively. The blastocyst formation rates were 54.2% (N=1127) and 65.3% (N=376) in LAH and NO-LAH groups, respectively (p<0.0001). Overall, 1123 and 374 blastocysts were biopsied and for 1072 and 363 of them it was obtained the genetic result (95.5% and 97.1%, NS) in LAH and NO-LAH groups, respectively. The percentages of transfereable blastocysts were comparable between the two groups: 40.2% (N=452) and 41.2% (N=154) in LAH and NO-LAH groups (NS), respectively. In total, 275 and 47 frozen embryo transfer were performed in LAH and NO-LAH groups, respectively. In LAH group, 281 blastocysts were transferred and 139 of them implanted (49.5%). In NO-LAH group, 48 blastocysts were transferred and 22 of them implanted (45.8%, NS). The miscarriage rate was the same in two groups (19.0%). Statistical analysis was performed using Sudent's t-test and Chi-squared test at the level of p<0.05

Limitations, reasons for caution: Although the incubator employed for the culture was the same in all analyzed embryos, the use of different culture media in the two groups could have introduced a bias in the study. The study has a retrospective design

Wider implications of the findings: Despite NO-LAH group includes younger women and higher fertilization and blastocyst formation rates compared to LAH group, the percentage of blastocyst available for transfer and the implantation rate were comparable. This outcome should reassure the operators about the LAH day-3 ZP opening in order to simplify the blastocyst biopsy procedure

Trial registration number: Not applicable

P-229 Can we predict aneuploidy or mosaicism considering blastocyst morphology?

A. Rodríguez-Arnedo[1], L. Herrero[2], L. Cascales[2], J.A. Ortiz[3], A. Bernabeu[4], J. Llácer[4], A. Llaneza[5], J. Ten[1], R. Bernabeu[4]

[1]Instituto Bernabeu, Embryology Dept., Alicante, Spain ;
[2]Instituto Bernabeu, Embryology Dept., Madrid, Spain ;
[3]Instituto Bernabeu, Molecular Biology, Alicante, Spain ;
[4]Instituto Bernabeu, Reproductive Medicine, Alicante, Spain ;
[5]Instituto Bernabeu, Reproductive Medicine, Madrid, Spain

Study question: Can we correlate blastocyst morphology with aneuploidy or mosaicism rates?

Summary answer: Blastocyst morphology: quality and expansion (percentage of trophectoderm (TE) hatching out of the zona) as well as maternal age are associated with aneuploidy and mosaicism.

What is known already: Preimplantation genetic testing of aneuploidies (PGT-A) has significantly grown in assisted reproduction treatments in the last decade. PGT-A aims to find the best euploid embryo, improving birth rates and allowing single embryo transfer. Embryo morphology is considered a predictor of implantation potential and several publications support a correlation between embryo development parameters with the chromosomic status of the embryo. Nonetheless, many "top-quality" embryos still turn out to be aneuploid after PGT-A. The aim of this study is to identify which embryo features are correlated with higher aneuploidy or mosaicism rates, and if it is possible to predict them.

Study design, size, duration: Retrospective database study performed between August 2017 and December 2018 including 162 PGT-A cycles and 499 biopsied embryos. Cycles with severe male factor (sperm samples concentrations under one million/mL) were excluded.

Participants/materials, setting, methods: Blastocyst quality was assessed according to Gardner and Schoolcraft score. Expansion was classified according to the percentage of Trophectoderm (TE) hatching out of the zona. Mosaic embryos with ≤25% aneuploid cells were considered euploid, and those with >50% were considered aneuploid.

Student's t, Pearson's chi-square and multivariate analysis (linear and binary logistic regressions) were used. Maternal age, embryo quality and expansion, day of biopsy and oocyte origin were introduced as confounding variables.

Main results and the role of chance: A total number of 310 blastocysts were biopsied on day-5 (62.1% out of 499). Three-hundred and twenty-eight blastocysts came from donor eggs cycles (65.7%) and 183 from frozen/thawed oocytes (36.7%). The diagnosed blastocysts (4.6% amplification failure) were classified in: 57.7% euploid, 13.7% euploid mosaic and 42.4% aneuploidy blastocysts. A tendency of lower aneuploidy rates as the blastocyst expansion increases was observed: 5-25% extruded TE: 47.7% aneuploidy, 25-50% extruded TE: 44.0% aneuploidy, >50% extruded TE: 32.6% aneuploidy, 100% extruded TE: 28.6% aneuploidy (p=0.052). Maternal average age also decreased as the blastocyst expansion increased (p=0.089). TE quality was found to be significative in predicting aneuploidy rate: 40.8% aneuploidy (TE A/B), 93.3% (TE C/D;

p=0.004). Maternal average age of blastocyst with A/B TE was lower than those blastocysts with C/D TE (29.8±9.8 vs 31.8±6.8 years), and also predictive for aneuploidy: p<0.001. TE quality (p=0.016) and maternal age (p=0.012) were found to be the most important variables predicting mosaicism. Logistic regression and multivariate aneuploidy risk score were performed. AUC was used to assess the predictive performance of the aneuploidy risk score: AUC=0.719 (95% CI 0.672-0.767). The same multivariate analysis was performed to predict euploid mosaic presence, and the AUC was 0.672 (95% CI 0.595-0.748).

Limitations, reasons for caution: Expansion and quality assessment is operator dependent and a potential source of subjective bias. Further studies focused on non-invasive embryo biomarkers for implantation potential to improve selection are warranted. PGT-A cannot yet be replaced by a genetic status statistical estimation, but it will be helpful in patient counselling.

Wider implications of the findings: Our results suggest a correlation between aneuploidy and blastocyst expansion/TE quality. This information might be helpful for patient counselling especially when PGT-A is not considered. Based on our findings, poor morphology and slow blastocysts from older patients carry a high risk for aneuploidies.

Trial registration number: no trial rnumber

P-230 Comparison of ploidy outcomes between grouped and single embryo culture in preimplantation genetic testing for aneuploidy (PGT-A) cycles.

S. Makieva[1], L. Pagliardini[1], L. Corti[1], A. Alteri[1], E. Papaleo[1], G. Cermisoni[1], A. Biricik[2], F. Spinella[2], F. Fiorentino[2], P. Viganò[1]

[1]IRCCS San Raffaele Scientific Institute, Obstetrics and Gynecology Unit, Milan, Italy ;
[2]Genoma, Molecular Genetics Laboratory, Rome, Italy

Study question: Does grouped embryo culture influence ploidy outcomes in PGT-A cycles?

Summary answer: PGT-A cycles performed using grouped embryo culture had 7.1% (p=0.02) higher euploidy rate compared to single embryo culture.

What is known already: Animal and human studies have shown that single embryos in culture lack the benefits of embryotrophic factors of group culture. In mice, single culture is associated with suboptimal neonatal outcomes while in humans grouped culture reduces embryo development rates, blastocyst quality and pregnancy rates. Thus, single culture may be detrimental to human embryos compared to grouped culture plausibly due to deprivation of paracrine signaling occurring between neighboring embryos. To date no study has assessed whether grouping embryos can influence their genetic composition and, hence, PGT-A outcomes.

Study design, size, duration: A retrospective analysis of 342 PGT-A cycles involving 1265 biopsied blastocysts performed between December 2014 and July 2019. The blastocysts were cultured either in groups of 1-6 (grouped culture; n=616) between December 2014 and March 2017 or individually (single culture; n=649) between May 2017 and July 2019, under similar conditions. The biopsy was performed between day 5 and 7 of development. The PGT-A diagnosis and other IVF outcomes were compared between the two groups.

Participants/materials, setting, methods: PGT-A cycles were performed at the IVF center of San Raffaele Hospital and included patients with an indication for advanced maternal age, recurrent implantation failure or a risk for a genetic disease. Grouped and single embryo culture involved a sequential system using culture media supplemented with SSS. All procedures were performed according to standard clinical practice. The PGT-A diagnosis was based on WGA+aCGH or NGS, which was predominantly performed at the genetics laboratory GENOMA.

Main results and the role of chance: The analysis of 1265 blastocysts revealed that grouped culture had a higher number of euploid (176/616; 28.5%; p=0.027) and lower number of mosaic (72/616;11.6%; p=0.025) blastocysts when compared to single culture (150/649;23.1% and 104/649;16% respectively). The number of grouped blastocysts biopsied on day 5 (261/616; 42.3%) was higher (p<0.0001) when compared to single blastocysts (166/649;26.9%). Moreover, single cultured blastocysts were more likely to be biopsied on day 6 (414/649;67.2%; p<0.0001) and day 7 (69/649;11.2%; p=0.0015) compared to grouped (366/616;49.6% and 49/616;7.9% respectively). Overall, the rate of euploidy in the 159 cycles in the grouped culture group was significantly higher compared to that in the 183 cycles of single group (28.66 ±29.19% vs 20.95 ±28.45%; p=0.002). No difference was observed in the rate of mosaicism. In the grouped culture cycles, the rate of day 5 biopsy was higher (38.73 ±18.89% vs 24.76 ±19.28%; p<0.0001) and of day 6 lower (52.39±29.07% vs 63.69 ±33.75%; p=0.0012) compared to single culture cycles. A generalised linear estimation corrected for variables known to be associated with ploidy according to the literature. Single embryo culture had 7.1% (13.2-1.1 %CI) lower euploidy rate compared to grouped embryo culture (p=0.02).

Limitations, reasons for caution: The study is limited by its retrospective design. The grouped embryo culture contained embryos cultured individually when the cycle had 1 embryo in excess. Cycles including at least 1 blastocyst with inconclusive diagnosis were included. The methodology used for assessment of chromosomal constitution may have introduced a bias.

Wider implications of the findings: Our findings suggest that grouped embryo culture may benefit PGT-A cycles by increasing the number of blastocysts diagnosed as euploid. This phenomenon could be attributed to the paracrine communication that may aid cell correction and free the blastocyst from aneuploid cells, which if persist may result in a mosaic diagnosis.

Trial registration number: non applicable

P-231 Suitability of 3D culture system with GrowDex for long-term culture of granulosa cells

E. Chmelíková[1], M. Sedmíková[1], M. Ješeta[2], P. Postlerová[1], R. Krejčířová[1], T. Krejčová[1], K. Havlíková[1], J. Žáková[2], P. Ventruba[2]

[1]Czech University of Life Sciences Prague, Department of Veterinary Sciences, Prague, Czech Republic ;
[2]Faculty of Medicine- Masaryk University Brno and University Hospital Brno, Department of Obstetrics and Gynecology, Brno, Czech Republic

Study question: Is it possible to increase the suitability of *in vitro* culture system for long-term granulosa cells cultivation using three dimensional (3D) culture with hydrocellulose GrowDex?

Summary answer: Using of 3D long-term culture system with GrowDex resulted in increasing of anti-apoptotic Bcl-2 and decreasing of pro-apoptotic Bax factors in porcine granulosa cells.

What is known already: Granulosa cells (GC) play important role in meiotic maturation of oocytes: by steroidogenesis, hormone activity regulation and prevention apoptosis. Apoptosis is regulated by pro- and anti-apoptotic factors; well-known of them are Bcl-2 and Bax. GC represent suitable model for *in vitro* culture, however when GC are cultured in monolayers they gradually lose their functions. 3D culture systems more closely mimic cell physiology environment and preserve interactions between cells. GrowDex hydrogel could be used as a matrix for 3D cultivation. GrowDex is a nanofibrillar cellulose hydrogel that forms fibres with length of several micrometres while their diameter is only 4-100 nanometres.

Study design, size, duration: In this study, porcine cumulus oocyte complexes (COCs) were matured in culture medium with GrowDex hydrogel as a matrix for 3D culture system (20 COCs; 4-well NUNC dish; 38°C; 5% CO_2). Conventional (2D) culture system was used as a control. After 48-hour culture matured oocytes were removed from COCs and GC were further cultured for 16 days. After pro-long culture, apoptotic factors (Bcl-2 and Bax) were determined in GC samples. Experiment was four times repeated.

Participants/materials, setting, methods: BCl-2 and Bax proteins were detected by blot immunodetection. The antigen-response signal to antibody was expressed on the basis of integrated optical density (IOD), which is equal to the average optical density and the area at the antigen-antibody response. In order to assess the amount of detected proteins, the IOD values were compared to the integrated optical density of non-cultured GC (RIOD=1). Statistical evaluation was performed using one-way ANOVA.

Main results and the role of chance: 3D culture system with GrowDex was shown to be more suitable for long-term culture of porcine granulosa cells as compared with conventional 2D culture system. Granulosa cells cultured in 3D culture system expressed pro-apoptotic factor Bax in significantly lower manner than those in 2D conventional system (RIOD = 0.3 vs. 0.73). On the contrary, the expression of the anti-apoptotic factor Bcl-2 was significantly higher in GrowDex culture system (RIOD = 2.31 vs. 1.61).

Limitations, reasons for caution: A limitation is the number of samples included and analysed in this study, which slightly reduced the power of statistical analysis.

Wider implications of the findings: 3D culture system with GrowDex could be tested for human granulosa cells when the long-term culture is required.
Trial registration number: Not applicable

P-232 Blastocyst re-expansion after warming is not predictive of implantation after euploid blastocyst transfer.

S. Mateo cuadros[1], M. Parriego[1], M. Solé[1], L. Coll[1], M. Tresanchez[1], F. Martínez[1], M. Boada[1], A. Veiga[1]

[1]Dexeus Mujer, Reproductive Medicine Service- Department of Obstetrics- Gynecology and Reproduction, Barcelona, Spain

Study question: Is blastocoel re-expansion a predictor of implantation in euploid blastocyst transfer?
Summary answer: Blastocoel re-expansion degree before embryo transfer does not correlate with implantation potential in warmed euploid blastocysts.
What is known already: Vitrification has improved the efficiency of embryo cryopreservation allowing the achievement of similar results after frozen/warmed embryo transfer compared to the fresh ones. In PGT cycles where a freeze-all strategy is needed, vitrification results have enabled the transition to blastocyst biopsy. Nonetheless, little is known about embryo characteristics after warming that can impact on implantation after euploid embryo transfer. Time between biopsy and vitrification, as well as time between warming and transfer, have been matter of study without conclusive results. Similarly, blastocyst re-expansion after warming has been proposed as a good prognosis parameter but without consensus in the literature.
Study design, size, duration: Prospective observational study of 113 euploid blastocysts replaced in single frozen-thawed embryo transfers performed from June to December 2019. Characteristics of the implanted and non-implanted blastocysts were compared. Blastocyst re-expansion index was the main variable analysed. Secondary variables were blastocyst morphology before biopsy, elapsed time between biopsy and vitrification and between warming and transfer.
Participants/materials, setting, methods: Blastocyst morphology included: inner cell mass (ICM) and trophectoderm (TE) grade (A, B or C, according to ASEBIR classification). Biopsied blastocysts were vitrified/warmed as described by manufacturer (Kitazato®). In order to assess the degree of blastocoel re-expansion, two pictures of the blastocysts were obtained: one taken immediately after warming (AW) and the second before transfer (BT). Re-expansion index was calculated as follows: diameter BT/diameter AW. Presence or absence of degenerated cells was also evaluated.
Main results and the role of chance: The mean maternal age was 35.5±6.1. Sixty-three of 113 blastocysts implanted (IR=55.8%). Implanted blastocysts exhibited significantly better ICM and TE quality than those that failed to implant (ICM A: 78.6% vs 21.4%, p=0.018; TE A: 80% vs 20%, p=0.012). Blastocoel re-expansion index ranged from 0.2 to 2.4 without differences between implanted and non-implanted blastocysts (1.3±0.3 vs 1.2±0.3 respectively; p=0.096). Degenerated cells after warming were detected in 39 blastocysts (34.5%). This feature was associated with an impaired implantation ability after embryo transfer (64.9% vs 38.5%; p=0.007). Elapsed time between biopsy and vitrification ranged from 0.2h to 7.2h with no differences between implanted and non-implanted blastocysts (1.9h vs 2.0h; p=0.938). Elapsed time between warming and embryo transfer ranged between 0.2h and 5.2h with no differences between implanted and non-implanted blastocysts (3h vs 3.2h; p=0.412).
Limitations, reasons for caution: These are preliminary results. For a deeper analysis controlling for potential confounding factors more data are needed. As unique SOPs were applied in a single IVF setting the time ranges included were limited.
Wider implications of the findings: Time to vitrification after biopsy, as well as time to transfer after warming can be adjusted according laboratory needs. When dealing with poor morphology blastocysts before vitrification and degenerated cells arise after warming, double embryo transfer might be proposed to selected couples.
Trial registration number: Not applicable

P-233 Expression of genes related to lipid metabolism in cells of the cumulus oophorus retrieved from patients undergoing assisted reproductive treatments.

S. Pizzasegale[1], A. Stendardi[1], T. Ruocco[2], F.L. Vellucci[1], F. Ietta[2], B. Fineschi[1], L. Gambera[1]

[1]A.G.I. Medica, Reproductive Medicine, Siena, Italy ;
[2]Università degli studi di Siena, Dipartimento di Scienze della Vita, Siena, Italy

Study question: is there a difference in the expression of some genes involved in lipid metabolism, between different categories of patients?
Summary answer: Some genes involved in lipid metabolism are differently expressed overall in patients with reduced ovarian reserve.
What is known already: Successful reproduction is dependent on the ovulation of competent oocyte, capable of undergoing fertilization and subsequent embryo and fetal development, and requires the co-ordinated growth and maturation of the oocyte and its somatic cells. Oocytes, but also cumulus cells are well known to contain lipid droplets, but not yet much is known about their role play during the maturation of the oocyte. Further studies are needed to understand the expression profile of those genes that regulate folliculogenesis especially during the last stages of follicular maturation that could be useful in identifying the lipids most involved during oocyte development.
Study design, size, duration: Present study included 16 IVF patients referred to our fertility Center A.G.I. Medica. Patients were subdivided into 3 groups according to the type of infertility: male infertility (control Group, n=7), Group 1 (G1, n=5) idiophatic infertility and Group 2 (G2, n=4) reduced ovarian reserve. The study was performed in cumulus cells obtained from cycles of intra-cytoplasmatic injection between March and October 2019.
Participants/materials, setting, methods: Following oocytes denudation, cumulus cells (CC) were collected and cumulated for each patient. CC were then washed and centrifuged and their number and viability was evaluated with Trypan Blue dye. In addition, mRNA extraction and retrotranscription was performed and through the Real Time Quantitative PCR were analyzed and quantified following genes: 11β-HSD 1, 11β-HSD 2, NR3C1 FASN, PNPLA2.
Main results and the role of chance: our data reported low viability of CC in the G2, which is also the group with the highest average age (39.5-2). These results suggest that the incidence of CC viability could predict the age-related decline in fertility. Molecular studies indicate that the expression of 11β-HSD-1 or 2 in the ovary depends on the prevailing hormonal environment and/or functional phenotype of cells. Compared to the control group our results indicate reduced levels of 11β-HSD-1 in G1 and G2 whereas 11β-HSD-2 is down regulated in G2 only. Considering the 11β-HSD-1/11β-HSD-2 ratio, which should increase physiologically during the ovulatory phase, this appears to have a clearly decrease especially in G2: this could mean that in this group an adequate conversion to cortisol does not take place. In addition in G2 the NR3C1 (glucocorticoid receptor) expression increase significantly compared to control; this could indicate a greater presence of receptor for low hormone levels as physiological feedback. PNPLA2 (involved in lipolysis) is significantly increased in G2, this could indicate a greater presence of free fatty acids. The FASN expression (involved in the synthesis of fatty acids) significantly increases in G1 and G2, compared to the control group and this could indicate an increased cellular metabolism.
Limitations, reasons for caution: Additional studies with an increased sample size in different clinical group will help to validate present results. In addition, it would be appropriate to evaluate the viability and expression of the lipid metabolism genes in CC of individual oocytes and not by cumulating them as we did in this study.
Wider implications of the findings: none
Trial registration number: None

P-234 Intracytoplasmic sperm injection versus conventional in vitro fertilization in non-male factor infertility. Interim analysis of a prospective randomized study

P. Fancsovits[1], Z. Kaszas[1], A. Nemes[1], K. Szedlak[1], A. Murber[1], D. Pocsi[1], A. Arokszallasi[1], J. Urbancsek[1]

[1]Semmelweis University, Division of Assisited Reproduction- Department of Obstetrics and Gynecology, Budapest, Hungary

Study question: Does intracytoplasmic sperm injection (ICSI) superior to conventional in vitro fertilization (IVF) as a method of fertilization in non-male factor infertility?
Summary answer: Conventional IVF resulted in higher fertilization rate while pregnancy and implantation rate was similar in IVF and ICSI cycles.

What is known already: ICSI is the most effective assisted reproductive technique to treat male infertility. However, it is also used for non-male factor indications, such as low number of oocytes or advanced maternal age. Thus, the incidence of ICSI fertilization method in fresh IVF cycles exceeds 70 % worldwide. However, data on the superiority of ICSI over conventional IVF in case of non-male factor infertility are still lacking.
Study design, size, duration: Data of interim analysis from an ongoing prospective randomized study performed in a university setting are presented here. Two hundred thirty two IVF cycles were randomized into ICSI or conventional IVF group between January 2018 and November 2019.
Participants/materials, setting, methods: Inclusion criteria: semen parameters suitable for conventional IVF fertilization, number of oocytes ≤4 and/or female age ≥40 years. Primary outcome was fertilization rate, secondary outcome was clinical pregnancy and implantation rates. Embryo development and quality (cell number, fragmentation, morphology score, rate of good quality embryos) on day 2 were also compared. Cycles with low oocyte number (A), advanced maternal age (B) and both factor together (C) were also investigated in a subgroup analysis.
Main results and the role of chance: Oocytes were fertilized by conventional IVF in 118 and by ICSI in 114 cycles. Fertilization rate was significantly higher in IVF group (IVF:62,7%, ICSI:50,8%; P<0,001). Clinical pregnancy rate was 24,5% (25/102) in IVF group and 18,8% (18/96) in ICSI group. The difference was not significant (P=0,326). Implantation rate was also similar in the two group (IVF:13,0%, ICSI:9,6%; P=0,270). Number of blastomeres (IVF:3,79±1,34, ICSI:3,85±1,14; P=0,596), embryo score (IVF:2,17±0,87, ICSI:2,21±0,78; P=0,495) and frequency of good quality embryos (IVF:29,4%, ICSI:26,9%; P=0,510) was comparable in the two group.

In cycles with ≤4 oocytes (Subgroup A) the fertilization rate, clinical pregnancy rate and implantation rate was similar in the two group. In case of advanced maternal age (≥40 years; Subgroup B) the fertilization rate was significantly higher in IVF group (IVF:64,1%, ICSI: 49,5%; P<0,001), but clinical pregnancy rate and implantation rate did not differ between the two fertilization method. In cycles where advanced maternal age was associated with low number of oocytes (Subgroup C), the fertilization rate (IVF:60,9%, ICSI:47,1%; P=0,043), clinical pregnancy rate (IVF:23,7%, ICSI:3,0%; P=0,013) and implantation rate (IVF:14,3%, ICSI:2,0%; P=0,018) was significantly higher in case of conventional IVF compared to ICSI.
Limitations, reasons for caution: This is an interim analysis of a prospective randomized study. Number of cases are low to draw a powerful conclusion on the effect of fertilization method on pregnancy an implantation rates.
Wider implications of the findings: Our data show a clear evidence that ICSI is not superior over conventional IVF in non-male factor infertility as conventional IVF resulted in higher fertilization rate. Furthermore, in case of advanced maternal age combined with low oocyte number, conventional IVF could be the most effective method of fertilization.
Trial registration number: ClinicalTrial: NCT03513913

P-235 Germline nuclear transfer to overcome mitochondrial disease and female infertility

M. Tang[1], A. Boel[1], R.R. Guggilla[1], B. Bekaert[1], P. De Sutter[1], B. Menten[2], F. Vanden Meerschaut[1], D. Stoop[1], P. Coucke[2], B. Heindryckx[1]

[1]Ghent University Hospital Ghent Fertility and Stem cell Team, Department for Reproductive Medicine, Gent, Belgium ;
[2]Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium

Study question: Can germline nuclear transfer (NT) technique serve as a novel reproductive approach to overcome mitochondrial DNA (mtDNA) diseases as well as female infertility?
Summary answer: The NT technique has a high potential to overcome mitochondrial disease transmission and certain forms of female infertility in human.
What is known already: Mitochondrial disease prevention may currently be possible using NT, such as spindle transfer (ST) and pronuclear transfer (PNT). These techniques involve transferring nuclear genome from an oocyte/zygote carrying mtDNA mutations to an enucleated donor counterpart with unaffected mtDNA. Besides, NT has also been considered for certain infertility indications, such as infertile women experiencing poor embryo development. This is mainly based on the rationale that replacing a low-quality cytoplasm with a competent one using NT, will improve oocyte competence, resulting in better IVF outcomes. Nevertheless, there are limited data on applying NT in human for overcoming mitochondrial diseases and female infertility.
Study design, size, duration: ST and recently proposed early (e)PNT were performed in the first patient using *in vivo* matured (n=5) and an *in vitro* matured (IVM) oocyte, respectively. Donated enucleated frozen-thawed *in vivo* matured oocytes with smooth endoplasmic reticulum aggregates (SERa) and a healthy fresh IVM oocyte (after ICSI and enucleation) served as cytoplast recipients, respectively. The second patient's spindles were transferred from fresh IVM oocytes (n=5) into enucleated frozen-thawed SERa oocytes from other patients, followed by ICSI.
Participants/materials, setting, methods: The first patient was a 30-year-old woman carrying a homoplasmic mtDNA mutation m.11778G>A, which is known to cause Leber's hereditary optic neuropathy (LHON) syndrome. The second patient was a 26-year-old woman experiencing two failed ICSI cycles characterized by almost all oocytes having fertilization failure. Finally, using B6D2F1 mice, we conducted RNA sequencing (RNA-seq) on PNT-blastocysts (n=3). Blastocysts from ICSI-oocytes (n=3) and unmanipulated oocyte pools (n=2) served as controls to assess safety concerns of NT technology.
Main results and the role of chance: For the patient harboring m.11778G>A mutation, PGD confirmed almost 100% homoplasmic mutation load in all embryos analyzed, which could not be used for embryo transfer. After ICSI, 5/5 of ST-oocytes were normally fertilized, 5/5 cleaved, and 1/5 progressed to the blastocyst stage (scored as 4AA); while 1/1 reconstructed ePNT-zygote reached morula stage. NGS data revealed that mtDNA carry-over in two trophectoderm biopsy samples from the ST-blastocyst and the ePNT-morula was 3.2%, 2.9% and 3.1%, respectively, Carry-over of remaining arrested ST-embryos was 2.9%. For the patient displaying fertilization failure, we first applied the mouse oocyte activation test to evaluate sperm activation potential of patient's partner. After injecting human sperm into mouse oocytes, more than 85% of mouse oocytes were activated (suspected oocyte-related problem). The application of assisted oocyte activation (AOA) resulted in only one oocyte fertilized (1/12), showing vague 2PN and no division further. Following ST and routine ICSI without AOA, 2/4 reconstituted oocytes were normally fertilized with 2PN, one showed 1PN, and the last one underwent immediate cleavage, and 3/4 subsequently cleaved further but did not progress to blastocysts. RNA-seq data showed that mouse PNT-blastocysts clustered very closely with ICSI controls, indicating that NT did not significantly change global gene expression.
Limitations, reasons for caution: The scarce availability of human oocytes donated for research purposes is a limiting factor to obtain more pre-clinical evidence of this new NT technology. In addition, molecular analysis of reconstructed embryos following NT is still required to verify the safety concerns.
Wider implications of the findings: Both ST and ePNT show the capacity to prevent the transmission of pathogenic mtDNA mutations of human, with minimal carry-over in the reconstructed embryos. Moreover, as a proof-of-concept, ST might be able to overcome fertilization failure in case of an oocyte-related activation deficiency causing failed fertilization after ICSI and AOA.
Trial registration number: Not applicable

P-236 Performance variability among Spanish oocyte cryobanks

G. Terzuoli[1], M.C. Magli[1], L. Maresca[1], D. Visone[1], A.P. Ferraretti[1], L. Gianaroli[1]

[1]S.I.S.Me.R., Reproductive Medicine Unit, Bologna, Italy

Study question: Do vitrified oocytes coming from different cryobanks have a different performance in the same oocyte donation program?
Summary answer: In our oocyte donation program using vitrified oocytes, there are significant, cryobank-dependent differences in the clinical performance of the thawed oocytes.
What is known already: Several studies show that the use of vitrified oocytes does not negatively affect the clinical pregnancy rate compared to fresh oocytes. Therefore, the import of vitrified oocytes from cryobanks has been adopted in many oocyte donation programs as a practical solution to the major problems related to the realization of this program. These include the difficulty of recruiting local donors, and the need to perform the screen tests requested by the European Directives on Tissues and Cells.
Study design, size, duration: This retrospective study includes 275 oocyte donation cycles from January 2016 to December 2019. We used vitrified oocytes

from four different cryobanks that were selected on the basis of their oocytes' availability. Laboratory data and clinical outcome were analyzed and compared among the four cryobanks, including oocyte survival rate, clinical pregnancy rate at the first transfer, and ongoing cumulative pregnancy rate. We also calculated the number of oocytes needed to have a baby born.

Participants/materials, setting, methods: The oocytes from the four cryobanks were used as follows. Cryobank bank 1, 67 cycles; cryobank 2, 22 cycles; cryobank 3, 116 cycles; and cryobank 4, 70 cycles. A minimum of 6 oocytes were allotted to each cycle and the same transfer policy was applied to all cycles. All thawing procedures were performed by experienced embryologists that strictly followed the given protocol and after a specific training provided in loco by each cryobank.

Main results and the role of chance: The characteristics of the recipients in the four groups were similar. No relevant differences among the four cryobanks were found in the survival rate (mean value 82.7%, range 79.08-89.4%). Conversely, the clinical outcome showed significant disparities with cryobank 1 having the lowest clinical pregnancy rate per thawing cycle at the first transfer (24.1%) in comparison with cryobank 3 (40.5%, P<0.025) and 4 (40%, P<0.05). The same trend was registered in the cumulative pregnancy rate (29.8% vs. 55.1%, P<0.005; and 51.4%, P<0.025 respectively). Clinical and cumulative pregnancy rates in oocytes from cryobank 2 were 22.7% and 36.3% respectively, but due to the low number of treated cycles, these data were not used in the statistical examination. To calculate the number of oocytes needed to have a baby born, the analysis of the data was restricted until December 2018. In all, the number of oocytes to be thawed to have a birth were 23.1 in cryobank 1, 21.2 in cryobank 2, 15.7 in cryobank 3 and 14.6 in cryobank 4.

Limitations, reasons for caution: The number of cycles in the four cryobanks was not homogenous.

Wider implications of the findings: The different quality of thawed oocytes from different cryobanks is not necessarily reflected by their survival, but by their developmental potential. Considering a tight control of thawing conditions, possible causes could be the donors' ovarian stimulation and/or the operator of the vitrification procedure, suggesting that oocyte viability is cryobank-dependent.

Trial registration number: not applicable

P-237 Cytoplasmic strings, who would say that they are not as bad as we thought?

M. Palma Rodríguez[1], C. Olmedo-Illueca[1], V.M. Chapero-Ciurana[1], D. Domingo-Fernandez-Vermejo[1], M. Barea-Gomez[1], L. Abad-De-Velasco[1], S. Royo-Bolea[1], I. Cuevas-Saiz[1]

[1]Hospital General de Valencia, Unidad de Medicina Reproductiva, Valenca, Spain

Study question: Is there any relation between cytoplasmic strings and embryo quality as well as implantation outcomes?

Summary answer: In this study correlation was found between presence or absence of cytoplasmic strings and implantation outcomes in addition to blastocyst quality.

What is known already: It has been described that, during *in-vitro* embryo development, trophectoderm (TE) cells project cytoplasmic extensions to the inner cell mass (ICM), known as cytoplasmic strings or filopodia. Cytoplasmic strings are mainly classified into two groups: Short strings, which are extended from the ICM to the mural TE and long strings, which are extended from the ICM, trough the blastocoelic cavity, to the polar ICM. According to bibliography, cytoplasmic strings are still present during expansion in poor quality blastocysts and related to poor media conditions.

Study design, size, duration: Presence/absence of cytoplasmic strings, type and the moment of development when were expressed (before/after or during blastocyst expansion) were retrospectively analyzed in 154 blastocyst that underwent fresh or frozen embryo transfer with known implantation data (KID). All data were collected from 2015 until 2019. Two groups were defined regarding KID+ or -. Furthermore, presence or absence of cytoplasmic string was also related to embryo quality. ASEBIR's embryo selection criteria was used to assess embryo quality.

Participants/materials, setting, methods: 131 patients underwent elective embryo transfer. 154 embryos were cultured at MIRI-TL® (Esco, Denmark) incubator using Continuous Single Culture Media (Irvine Scientific, EEUU). To set correlation between presence/absence of cytoplasmic strings and KID+/-, *Chi-Squared* test was performed. To determine correlation between type and when strings were expressed with KID+/-, ANOVA test was used. Correlation between presence/absence and embryo quality was studied using *Kruskal-Wallis* test. All statistics were performed using SPSS© v22.0 (IBM, EEUU) software (p<0,05).

Main results and the role of chance: Out of 154 embryos, 123 showed cytoplasmic strings. 66,3% of them were KID+ and 56,7% were KID-. 31 embryos did not show any cytoplasmic strings; in this case 16,7% were KID+ and 14,3% were KID-. Significant differences were found (p=0,021) between presence or absence of cytoplasmic strings groups. Regarding type and moment when strings were expressed, no correlation was found between those two variables and implantation outcomes (p=0 ,742 and p=0,585, respectively). According to embryo quality and presence of cytoplasmic strings: 62,3% of embryos were A quality, 25,5% B quality, 10,2% C quality and 2% D embryos. However, in the absence of cytoplasmic strings: 38,5% were A quality, 53,8% B quality and 7,7% C quality. Significant differences were registered between these two groups (p=0,049).

Limitations, reasons for caution: To confirm if presence of strings in the expanded blastocyst is not related with a poor embryo quality (C and D classification in ASEBIR's embryo selection criteria) and that could be a good embryo implantation predictor, further studies should be carried out.

Wider implications of the findings: According to these findings and conversely to previous bibliography, the presence of cytoplasmic strings could have positive implication on embryo development and implantation outcomes. This filopodia could provide cells from ICM, which is mitotically active, to TE, which is mitotically less active and more differentiated.

Trial registration number: Not applicable

P-238 Clinical pregnancy in euploid blastocyst transfer is correlated to early cleavage and blastulation morphokinetic parameters

C. Jacobs[1], M. Nicolielo[2], L. Cremonesi[2], E.L.A. Motta[3], J.R. Alegretti[2], A. Lorenzon[4]

[1]Huntington Medicina Reprodutiva, Embryology, Sao Paulo, Brazil ;
[2]Huntington Medicina Reprodutiva, Embryology, São Paulo, Brazil ;
[3]Huntington Medicina Reprodutiva / Federal University of São Paulo, Clinical Director / Associate Professor, São Paulo, Brazil ;
[4]Huntington Medicina Reprodutiva, Research & Development, São Paulo, Brazil

Study question: What are the differences to achieve clinical pregnancy correlated to morphokinetic parameters with euploid embryos?

Summary answer: Euploid blastocysts that results in clinical pregnancy achieved time to 5 (t5), to 8 cells (t8) and blastulation (tB) at an earlier point in time.

What is known already: Embryo aneuploidy is known as the main cause of embryo implantation failure. The next-generation sequencing analysis (NGS) of blastocysts biopsied cells is the current most advanced technique to diagnose embryo genetic constitution allowing the selection of an euploid embryo for transfer. Beside the ploidy status, the morphokinetic evaluation for embryo selection can enhance the chance to achieve an ongoing pregnancy. The rapid spread of the time-lapse technology increased the demand of algorithms to predict embryo potential. However, intrinsic differences in each laboratory should be taken into consideration before applying global algorithms in routine before validation.

Study design, size, duration: Retrospective cohort study analyzing the morphokinetic parameters of 227 euploid biopsied embryos (NGS) from autologous cycles (n= 132 patients) with known implantation outcome (single embryo transfer: positive or negative gestational sac (GS) and fetal heartbeat (FHB), double embryo transfer: negative or two GS and FHB). Data was collected between December 2017 and December 2019 in a private IVF center.

Participants/materials, setting, methods: Blastocyst biopsied embryos from patients undergoing IVF with clinical indication for PGT-A were included. All oocytes were fertilized by ICSI and cultured in time-lapse system incubator (Embryoscope Plus, Vitrolife). Embryos were analyzed for the following morphokinetic parameters: time of pronucleous fading (tPNf), time to 2-cell(t2), time to 3-cell (t3), time to 4-cell (t4), time to 5-cell (t5), time to 8-cell (t8) and time to blastulation (tB). Embryos were graded according to Gardner's morphology parameters.

Main results and the role of chance: Two-hundred twenty-seven blastocysts were analyzed: 165 single embryo transfers (sET) and 62 embryos from 31 double embryo transfers (dET). From those, 119 blastocysts resulted in positive clinical pregnancy (49%, 81 from sET and 38 from dET) and 108 in negative (51%, 84 from sET and 24 from dET). Maternal age was similar between positive and negative clinical pregnancy (38,43 ± 3,15 versus 38,31 ± 4,07, p=0.9090). Morphokinetics parameters were earlier in euploid embryos that achieved clinical pregnancy in three time-points: t5, t8 and tB (t5: 48,55 ± 6,42 versus 50,46 ± 6,63, p=0.028; t8: 56,93 ± 8,43 versus 60,81 ± 9,57, p=0.001 and tB: 106,58 ± 9,71 versus 109,64 ± 11,05, p=0.023). Regarding other parameters analyzed, all earlier time-points were similar between positive and negative clinical pregnancy (tPNf: 23,11±3,76 versus 23,43±3,62, p=0.278, t2: 26,32±3,56 versus 26,66±2,99, p=0.239; t3: 36,84±4,29 versus 37,16±3,73, p=0.180; t4: 38,11±4,92 versus 38,56±3,50, p=0.162). Morphology grades for positive and negative clinical pregnancy were different between good quality embryos (grades A and B – 86% versus 73%) and poor quality embryos (at least one grade C – 14% versus 27%, p=0.021). Statistical significances were calculated using chi square or t-test as appropriate.

Limitations, reasons for caution: This is an observational study based on a retrospective database analysis, limited to biopsied embryos, generated by a population with PGT-A indication.

Wider implications of the findings: Our study highlights differences in morphokinetic parameters in euploid blastocysts that achieved clinical pregnancy. Since the differences in these parameters were independent from aneuploidy status, they represent potential time-points to be better analyzed in laboratory algorithms, reinforcing the potential use of morphokinetics as a tool for non-invasive embryo selection.

Trial registration number: Not applicable

P-239 The effectiveness of two cycles with single embryo transfer (fresh + frozen) versus one fresh double embryo transfer in women treated in egg donation program

S. Herzberg[1], S. Kraus[1], N. Schachter-Safarai[1], G. Karavani[1], A. Simon[1], A. Ben-Meir[1]

[1]Hadassah Ein Cerem- Jerusalem, Obstetrics and Gynecology, Jerusalem, Israel

Study question: What is the effectiveness of two cycles of Single Embryo Transfer (SET) versus Double Embryo Transfer (DET) in egg donation IVF treatment.

Summary answer: Implantation rate in two cycles of SET is similar to DET in egg donation IVF treatment. Multiple pregnancy rate is significantly higher in DET.

What is known already: The practice of transferring multiple embryos results in a high incidence of multiple births. Several studies on autologous IVF treatment have shown that two cycles of SET compared to DET result in a significant decrease of multiple pregnancy with similar pregnancy rates. Several factors can influence the results, including: the freezing and thawing process and an endometrial receptivity impairment secondary to the ovarian hyperstimulation. In egg donation we effectively neutralize the possible effect of exposing the endometrium to high levels of estrogen. This allows us to examine more clearly whether differences between fresh embryo and frozen-thawed transfers are present.

Study design, size, duration: A retrospective cohort study conducted at two IVF units in the years 2010-2019.

The patients were divided into 2 groups: Group A - DET - transferring of two fresh embryos in one cycle, Group B - SET - transferring of a single fresh embryo and later a second single frozen thawed embryo transfer in another cycle. Implantation rate, multiple pregnancy rate and obstetrical outcomes were compared between the two groups.

Participants/materials, setting, methods: Women treated with oocyte donation and who had at least two A-grade embryos were included in the study. The donors were healthy women 25-32 years. The endometrial preparation of the recipient women included estradiol for two weeks. On the oocyte retrieval day, the recipient began treatment with progesterone. The recipients were treated with Long-acting GnRH agonist, to prevent unplanned ovulation.

Main results and the role of chance: 251 women met the criteria set for the study. 168 women were included in the DET group and 81 women were included in the SET group. The two groups were similar in the demographic and clinical characteristics. Implantation rate was 32.3% in the SET group and 39.3% in the DET group, without significant difference (OR of 0.74, 95%CI: 0.48-1.13; p=0.16). However, multiple pregnancy rate was significantly higher in the DET group compared to the SET group (37.5% Vs 0%; p= 0.006), Moreover, preterm birth rate was 32.3% in the DET group compare to 13.9% in the SET group with OR of 2.94 (95%CI: 1.12-7.7; P value=0.03). Other obstetrical parameters were without significant difference between the two groups.

The similar implantation rate following two cycles of SET compared to DET in egg donation treatment means that the impaired endometrial receptivity following the ovarian hyperstimulation in autologous IVF treatment is not the main factor to explain this finding. It may support the existing information that the transfer of frozen-thawed embryo has similar success rates as the transfer of fresh embryo.

Limitations, reasons for caution: The retrospective design of our study makes it more prone to bias.

Wider implications of the findings: This study can give us an insight on the factors that contribute SET, DET outcomes in autologous and egg donation treatments. In addition, the study results support the use of elective SET in egg donation program in order to decrease the risk of multiple pregnancy without decreasing implantation rate.

Trial registration number: Not applicable

P-240 A novel microfluidic-based organoid mimicking the oviduct environment in vivo promote the embryo development via reducing cellular reactive oxygen species (ROS) level

M. Wang[1], B. Zhang[1], L. Jin[1]

[1]Tongji Hospital- Tongji Medical College- Huazhong University of Science and Technology, Reproductive Medicine Center, Wuhan, China

Study question: Could a microfluidic-based oviduct organoid promote the embryo development?

Summary answer: A microfluidic-based organoid mimicking both anatomical and biochemical environment of oviduct in vivo could promote the embryo development via reducing cellular ROS level.

What is known already: In current methods, embryos were cultured in petri-dish under substantially static conditions, conversely during the most of time, in vivo pre-implantation embryos experience dynamic micro-environments. In addition, the processes of fertilization and development from zygote to cleavage embryo were completed in the oviduct. Consequently, oviduct is extremely significant for pre-implantation embryo. However, due to the intra-abdominal anatomical location, it is difficult to access an available oviduct model for embryo development at present. Microfluidic chips are widely applied in various walks of life due to the extraordinary characteristics. Therefore, a microfluidic-based oviduct organoid was designed for embryo culturing.

Study design, size, duration: The microfluidic devices were fabricated following standard rapid prototyping methods. Mouse primary oviduct epithelial cells and embryos were obtained from ICR, and then identified by anti-Keratin immunofluorescence staining. Mouse embryos were cultured in chip and in dish separately and cellular ROS level were measured.

Participants/materials, setting, methods: The PDMS-PDMS devices were fabricated by multi-layer soft lithography technology. Oviduct epithelial cells and embryos were obtained from ICR. Mouse embryos were separated into two groups for further development. In microfluidic group, embryos were co-cultured with oviduct epithelial cells in the microfluidic-based organoid, and in control groups were cultured in traditional dish. The numbers of embryos in different stages were recorded, and then retrieved and exposed in H2DCFDA to measure cellular ROS level.

Main results and the role of chance: The novel microfluidic-based organoid in this study was able to mimic oviduct environment in terms of both anatomy and biochemistry of fallopian tubes. The oviduct-on-chip models were with a length of 2 cm and a diameter of 1 cm, which were in accordance with the anatomical characteristics of the oviduct in vivo. When the microfluidic chips were connected to the computer control system, the liquid can flow into the micro-channels through the inlets to outlets at a slow speed. Mouse primary oviduct epithelial cells were obtained and identified by immunofluorescence staining, and then loaded into the chip to provide a suitable biochemical environment. The levels of embryo ROS in microfluidic groups were statistically significant lower than that in control group ($P < 0.001$). In order to explore the effect of different conditions on the development of embryos, fertilization rate,

4-cell embryo rate, 8-cell embryo rate and high quality embryo rate were calculated. Compared with control group, the rates of different stages embryos in microfluidic group were higher, although there were no significant differences (P=0.159, 0.246, 0.823, 0.258 respectively).
Limitations, reasons for caution: The reduction of ROS concentration can definitely benefit to the embryo quality. However, the gold standard of embryo quality is DNA integrity of euploid embryo. In addition, mouse oocytes and sperms were used instead of human due to ethical requirements. More experiments are needed using human tissue.
Wider implications of the findings: This organ-on-chip device allows the probability of mammalian embryo culture in a microfluidic-based manner. Further development might promote the combination of this novel embryo development methods and clinical IVF practice, and enhance the cumulative clinical pregnancy rate.
Trial registration number: not applicable

P-241 Universal warming protocol for vitrified blastocysts: The "coming-out" of "off-label" use of warming kit brands

L. Parmegiani[1], C. Garello[2], S. Canosa[2], F. Granella[2], F. Evangelista[2], G. Monelli[2], D. Guidetti[2], F. Bongioanni[2], M. Filicori[3]

[1]GynePro Medical, Reproductive Medicine Unit, Bologna, Italy ;
[2]LIVET Srl, LIVET Srl, Torino, Italy ;
[3]GynePro Medical NextClinics International, Reproductive Medicine Unit, Bologna, Italy

Study question: Is it possible to use warming kit brands "off-label" to efficiently warm vitrified blastocysts?
Summary answer: Different vitrification and warming kits can be combined without affecting survival and implantation rates.
What is known already: Embryos are routinely vitirified using different commercial brands of ready-to-use vitrification solutions which differ only slightly in their composition. Every brand of vitrification kit requires the use of its own warming kit. However, it is useful for Assisted Reproduction centres to have alternative options for carrying out any procedure. The application of "Universal Warming" through the combination of different vitrification/warming kits can offer this flexibility, but this action may have potential legal consequences for AR centers. This study aims to give scientific validation to this common but "off-label" and thus often covert usage.
Study design, size, duration: Prospective randomized study. Vitrified blastocysts were randomized at warming. Group randomization 1:1:1 via www.random.org. Total number of vitrified/warmed blastocysts: 231. Duration: January – October 2018.
Participants/materials, setting, methods: 107 blastocysts from patients' own oocytes, 124 blastocysts form donors. Each patient's/ donor's embryos were vitrified with Kitazato Vitrification Kit (Japan) and warmed with three different warming kits: i) Kitazato, ii) Sage - Coopersurgical, Denmark, iii) Irvine -Fujifilm USA . Group names: KK, KS, KI, (patient's blastocysts); eKK, eKS, eKI (donor's blastocysts). Primary outcome measure: cryo-survival rate (embryos surviving/embryos warmed). Secondary outcome measure: embryo implantation rate (gestational sacs/embryos transferred).
Main results and the role of chance: Cryo-survival rate was statistically comparable between the study groups: KK 97.9% (47/48), KS 96.5% (53/55), KI 100% (47/47), eKK 97.5% (39/40), eKS 100% (37/37), eKI 95.7% (45/47). Embryo implantation rate was comparable between groups: KK 40.4% (19/47), KS 43.4% (23/53), KI 48.9% (23/47), eKK 46.1% (18/39), eKS 35.1% (11/37), eKI 46.7% (21/45).
Limitations, reasons for caution: These results need to be confirmed with larger studies investigating different "off-label" combinations of vitrification and warming kits.
Wider implications of the findings: This study confirms that the combination of different kits for vitrification/warming permits efficient survival and implantation, irrespective of brand, cryoprotectants and basic medium in the kits. This result supports the "off-label" use of different combinations of vitrification and warming kits, allowing practitioners worldwide to "come-out" of the closet.
Trial registration number: Not Applicable

P-242 Embryonic development and cytogenetic evaluation of apronuclear zygotes after 18 to 20 hours of in vitro fertilization

D. Freitas[1], C. Teixeira[1], A. Almeida[1], L. Queiroz[1], R. Carvalho[2], T. Oliveira[2], C. Santos[2], T. Abreu[2], M. Niag[2], G. Coelho[3]

[1]IVIRMA Brazil- Clinic IVI Salvador, IVF LAB, Salvador, Brazil ;
[2]Universidade Federal do Recôncavo da Bahia., Medicine, Santo Antonio de Jesus, Brazil ;
[3]IVIRMA Brazil- Clinic IVI Salvador, Clinical director, Salvador, Brazil

Study question: How is the embryonic development, cytogenetic constitution and blastocyst rates of apronuclear zygotes presenting two polar bodies ("0PN- pronuclei / 2CP- polar body")?
Summary answer: Apronuclear zygotes with two polar bodies ("0PN / 2CP") can be euploid and have appropriate embryonic development.
What is known already: The fertilization process involves ordered morphological changes that affect the gametes, cellularity, allowing to identify failures or changes in the zygote. A fertilization assessment comprises complex and dynamic classification systems for embryo morphology at various stages of development, which impact on embryo selection. The average observation time for fertilization is 16 to 22 hours. The interpretation of the absence of pro-nuclei (0PN) in zygotes with 2 polar body and the use of these zygotes in IVF has been a topic of debate. Cytogenetic analyzes reveal that a significant subset of these zygotes is diploid and with competent development.
Study design, size, duration: This is a descriptive retrospective, cross-sectional study. A convenience sample was used, covering the analysis of medical records of couples diagnosed with infertility, who underwent treatment at an assisted reproduction clinic in Salvador, Bahia, Brazil. This work was developed during the period from January to December 2019.
Participants/materials, setting, methods: This study was carried out through the analysis of medical records of couples submitted to assisted reproduction techniques for conjugal infertility. The analysis included 309 cycles of couples submitted to in-vitro fertilization and who had at least one apronuclear zygote. These analyzed embryos were cultured using sequential media until blastocyst (D5 / D6). Fertilization was evaluated between 18 and 20 hours after ICSI (Intracytoplasmic Sperm Injection) of 2642 MII oocytes (in metaphase II).
Main results and the role of chance: 2129 oocytes fertilized from 2642 oocytes MII injected, performing a fertilization rate of 80.58%. From these fertilized eggs, 295 were apronuclear, with the presence of 2 polar bodies (0PN / 2CP), representing 13.85% of the total number of zygotes. Among the 295 0PN / 2CP zygotes, a group of 36 embryos, belonging to 15 cycles of patients with an average age of 36.72 years, had their development monitored, since they would be submitted to cytogenetic study. 24 blastocysts were generated, with a blastocyst rate of 66.67%, being submitted to PGT (Preimplantation Genetic Testing) by NGS (New Generation Sequencing). Among the 24 embryos analyzed, 13 embryos (54.17%) had a normal karyotype, and 11 embryos (45.83%) had an abnormal result. Of the 13 euploid blastocysts, only 9 embryos have already been transferred and 3 of these embryos have implanted, which gives an implantation rate of 33.33%.
Limitations, reasons for caution: The reduced number of embryos analyzed in this study does not allow the establishment of more precise correlations between analyzed variables. This study will be complemented with new cycles to increase the investigation of the apronuclear zygotes (0PN / 2CP).
Wider implications of the findings: Embryos from apronuclear zygotes tend to be discarded due to erroneous attribution of ploidy based on numbers of pronuclei. Studies that evaluate the development in blastocysts and the genetic evaluation, demonstrate that many apronuclear zygotes present diploid, resulting in pregnancies with the birth of healthy babies.
Trial registration number: not applicable

P-243 Is the embryo utilization rate different after conventional IVF or ICSI in sibling oocytes?

I. Mateizel[1], P. Drakopoulos[1], H. Tournaye[1], G. Verheyen[1]

[1]UZ Brussel, Centre for Reproductive Medicine, Jette- Brussels, Belgium

Study question: Is the embryo utilization rate affected by the insemination procedure (conventional IVF or ICSI) in patients with split insemination of sibling oocytes?
Summary answer: Embryo utilization rates are similar after IVF and ICSI in sibling oocytes cycles.

What is known already: The use of ICSI in assisted reproduction has increased exponentially since its clinical introduction in 1991. Initially developed for severe male factor infertility, it is now offered for all causes of infertility, despite lack of data to justify this approach. Few studies have addressed the issue of the optimal insemination technique, with conflicting results and possible biased by the inclusion of women with poorer prognosis in the ICSI group. Hence, the ideal population to study the effectiveness of each insemination method, considering also the ovarian response, would be patients undergoing IVF and ICSI within the same cycle.
Study design, size, duration: The aim of our retrospective study was to compare the embryological outcome between IVF or ICSI insemination. All consecutive patients undergoing IVF versus ICSI procedure in their first ovarian stimulation cycle (only antagonist protocol) between 2009 and 2014 were included. Only patients with fresh semen samples fulfilling the minimum criteria for IVF were considered.
Participants/materials, setting, methods: Following ovarian puncture, the sibling cumulus–oocyte-complexes (COCs) were randomly allocated to either IVF or ICSI procedure. In cases of an uneven COC number, the extra COC was included in the ICSI arm. The embryos were cultured in sequential media until day 3 or day 5/6. The main outcome was the embryo utilization rate per fertilized oocyte (total number of embryos transferred and cryopreserved divided by the number of 2PN).
Main results and the role of chance: A number of 418 patients were included and divided according to their ovarian response in: poor (≤ 3 COC retrieved, suboptimal (4-9 COC), normal (10-15 COC) and high (>15 COC) responders.The median age [interquartile range (IQR)] was 32 (29-36), while the number of oocytes retrieved per insemination method was 5 (3-7). Fertilization rates defined as the ratio of 2PN per number of COCs were significantly higher in ICSI (67.5 vs. 54.1, p<0.001). However, embryo utilization rates per 2PN were similar between oocytes inseminated with IVF and those inseminated with ICSI (53.8% vs. 57.4%, p value =0.3). In order to assess the effect of the insemination technique on embryo utilization rates after accounting for several confounders (age, BMI, cause of infertility, initial stimulation dose, number of COCs), a regression model with estimation by generalized estimating equations (GEE) was used. According to GEE, the insemination method was not significantly associated with embryo utilization rate (coefficient 3.8, 95% CI -1.3 to 9, p=0.14). Also when considering subpopulations according to ovarian response, embryo utilization rate after IVF and ICSI were comparable (coefficient 3.4, 95% CI -1.8 to 8.5, p=0.2).
Limitations, reasons for caution: The retrospective nature of the present study can be considered as a limitation.
Wider implications of the findings: The robust design of our study, allowing each patient to serve as its own control, provides reassuring evidence that embryo quality is not impaired by the insemination method used.
Trial registration number: B.U.N.143201939166

P-244 A comparison of live birth and multiple pregnancy rates when 1 or 2 low quality blastocysts are transferred in a frozen cycle, stratified for age

S. Aldhaheri[1], S. Arab[2], A. Baeghiesh[1], M.H. Dahan[1]

[1]McGill University, Obstetrics and Gynecology, Montreal, Canada ;
[2]McGill University, Gyncecologic and Reproductive Endocrinology and Infertility - Obstetrics and Gynecology Department, Montreal, Canada

Study question: What are the in-vitro fertilization (IVF) outcomes in patient with Gardner's grade BB or lower frozen blastocysts transfers and the effect on multiple pregnancy rates?
Summary answer: If age was <40 but not ≥40, the multiple pregnancy rates were significantly higher in double embryo transfer (DET) than single embryo transfer (SET) cycles.
What is known already: Most studies have investigated outcomes based on high quality blastocyst transfer or a combination of good and poor quality blastocysts. Outcomes among women with only Gardner's grade BB, BC, CB and CC frozen embryos transferred (FET) are not well known. The outcomes stratified for age with low quality frozen blastocysts has also not been investigated, nor has the role of transferring two poor grade blastocysts on multiple pregnancy rates. Our study will be the first one to look at the multiple pregnancy and live-birth rates after transferring one or two Gardner's grade BB or lower quality frozen blastocysts.
Study design, size, duration: A retrospective cohort study including 1104 FET cycles was performed. All frozen Day 5 or 6 blastocyst of Gardner's grade BB or lower quality blastocysts transferred between January-2008 to December-2019 at an Academic Fertility center were included. 189 cycles were egg donor. Data was stratified for age, autologous or donor embryo and embryo quality. In the case of a double embryo transfer quality was based on the best grade of the two embryos.
Participants/materials, setting, methods: 96 cycles were in patients >40-years of age and 819 cycles in women <40 years of age, at collection. Clinical pregnancy (CP), multiple pregnancy (MP) and live birth rates (LBR) per cycle were compared between SET (n=969) and DET (n=135) in each group. Clinical pregnancy and multiple pregnancy rates were compared between SET and DET per cycle in grade BB (n=894), BC (n=86), CB (n=52) and CC (n=72) groups individually.
Main results and the role of chance: In SET group, Gardner's grade BB blastocysts had higher pregnancy 48% (P=0.000), CP 34% (P=0.000) and LBR 19% (P=0.059) in comparison to Gardner's grade BC, CB and CC single embryo transfers. Within BB FET cycles, MP rate was significantly higher when transferring two vs. one (5.9 vs. 1.9, P =0.009). Transferring two Gardner's grade CB, BC or CC embryos did not increase the multiple pregnancy rate **(**P=0.78)**.** If age at egg collection was ≥40 no difference was found in CP (11.1 vs. 11.7, P =0.9), MP (0 vs. 0) and LBR (6.3% vs. 0%, P=0.13) when SET or DET was performed. If age at egg collection was <40(N=819) the MP rate was significantly higher in DET than SET (6.75% vs 1.63%, P=0.004) even though, age at collection (37 vs. 33years, P=0.001) and transfer (39 vs. 35years, P=0.001) were significantly older, respectively. However, no difference was found in CP and LBR, overall. Among egg donors (N=189), there were no difference in CP (25.9% vs. 29.62%, P=0.68), MP (3.12% vs.7.4%, P=0.28) and LBR (11.72% vs. 22.2%, P=0.137) between SET and DET. Among egg donors the LBR for Gardner's grade BB, BC, CB and CC were (8.2%, 6.3% 9% and 0%), respectively (P=0.74).
Limitations, reasons for caution: This is a retrospective cohort study with the inherent risk of undetected bias. The small number of cycles in women ≥40 years of age at collection should be considered.
Wider implications of the findings: Among women with grade BB or lower blastocyst at ≥40 years-of-age transferring 2 did not increase the MP rates. In women <40 years-of-age transferring 2 BB blastocysts increased the MP rate, but may fall into acceptable limits. MP rates did not increase when transferring two CB, BC or CC blastocysts.
Trial registration number: MUHC REB arsu6002/2020-6228

P-245 Assessment of molecular determinants underlying recurrent embryo developmental arrest and value of assisted oocyte activation as a treatment option

A. Boel[1], D. Bonte[1], A. Cardona Barberan[1], V. Thys[1], D. Stoop[1], P. De Sutter[1], P. Coucke[2], F. Vanden Meerschaut[1], B. Heindryckx[1]

[1]Ghent University Hospital, Ghent-Fertility And Stem cell Team- Department for Reproductive Medicine, Ghent, Belgium ;
[2]Ghent University, Center for Medical Genetics Ghent- Department of Biomolecular Medicine, Ghent, Belgium

Study question: Can recurrent embryo developmental arrest be linked to specific molecular defects and be overcome by assisted oocyte activation (AOA)?
Summary answer: Applying stringent inclusion criteria, AOA did not show beneficial effects in our patient cohort, for which the molecular cause of embryo arrest remains mainly unexplained.
What is known already: Recurrent embryo developmental arrest has recently been linked to genetic defects in maternal effect genes. Treatment options for these patients are limited, however, assisted oocyte activation (AOA) has been proposed. AOA is routinely used to overcome fertilization failure after ICSI and involves the artificial induction of Ca^{2+} rises in the oocyte. The lack of Ca^{2+} oscillations, normally evoked by the sperm factor phospholipase C zeta (PLCζ), has been suggested to both contribute to failed fertilization, as well as to post-fertilization processes, such as embryo arrest or inferior blastocyst formation.
Study design, size, duration: A prospective study was performed between January 2018 and August 2019, including 11 couples, presenting with embryo

arrest or inferior blastocyst formation. Patients were only included when they underwent at least two fresh ICSI cycles. Furthermore, high fertilization rates (≥60% 2PN or zygote) with a minimum of 10 zygotes but low blastocyst rates (≤15% of at least early blastocyst quality on day 5) were required.

Participants/materials, setting, methods: All couples underwent a fresh AOA cycle ($CaCl_2$ injection and ionomycin exposure (2x)). Both partners donated a saliva sample for genetic screening of six MEGs (female) and *PLCZ1* (male). Except for one lesbian couple, the Ca^{2+} oscillatory patterns induced by the male patient's spermatozoa were examined through mouse oocyte Ca^{2+} analysis (MOCA). The mean maternal age was 33.4 years ± 3.4 years. Most couples suffered from male factor infertility (n=3) or unexplained female infertility (n=3).

Main results and the role of chance: In total, 11 AOA cycles were performed and compared to 31 previous routine ICSI cycles. The overall fertilization rate after AOA application was 70.1% (96/137) which was similar to the fertilization rate of 74.8% (237/317) after conventional ICSI. Blastocyst rates did not significantly increase after AOA (9.4%, 9/96) compared to the rates obtained in previous ICSI attempts (9.3%, 22/237). Furthermore, pregnancy rates were not significantly different between AOA (28.6%, 2/7) and conventional ICSI (36.8%, 7/19). Previous conventional ICSI cycles resulted in one miscarriage, two biochemical pregnancies, and four healthy singletons. Regarding the 11 AOA cycles, one embryo transfer resulted in an ectopic pregnancy, while the other pregnancy is still ongoing.

For all male patients, MOCA displayed Ca^{2+} oscillatory patterns with high Ca^{2+} spike frequencies, comparable to controls, confirming a normal oocyte activating capacity. Sequencing of the selected MEGs (*NLRP2, NLRP5, NLRP7, TLE6, PADI6, KHDC3L*) in female and *PLCZ1* in male patients revealed several variants, most of which were categorized as benign and likely benign. No *PLCZ1* pathogenic mutations were identified in the ten assessed male individuals. However, amongst the eleven tested female individuals who underwent AOA, we identified three unique heterozygous variants of uncertain significance.

Limitations, reasons for caution: Our study, showing that AOA is not able to overcome embryo developmental problems, which is in contrast with earlier findings, is limited by its small sample size and displays therefore only preliminary results. Genetic screening targets are too restricted to identify a genetic cause underlying the phenotype for all patients.

Wider implications of the findings: Strong evidence for AOA to overcome impaired embryonic development is still lacking, therefore, embryo arrest should not be an indication for AOA application. Genetic screening on a routine basis might be valuable to improve clinical management.

Trial registration number: NA

P-246 Is mosaicism and its characteristics influenced by the method of trophectoderm-biopsy technique employed in PGT-A cycles? Genetic and reproductive outcomes after pulling versus flicking.

L. Herrero Grassa[1], M. Aparicio Gonzalez[1], L. Cascales Romero[1], J.A. Ortiz[2], J.C. Castillo[3], C. García-Ajofrin[4], J. Ten[5], R. Bernabéu[3]

[1]Instituto Bernabéu Madrid, IVF Laboratory, Madrid, Spain ;
[2]Instituto Bernabéu Biotech, Molecular Genetics Laboratory, Alicante, Spain ;
[3]Instituto Bernabéu Alicante, Ginaecology, Alicante, Spain ;
[4]Instituto Bernabéu Madrid, Gynaecology, Madrid, Spain ;
[5]Instituto Bernabéu Alicante, IVF Laboratory, Alicante, Spain

Study question: Are pulling and laser (over)exposure influencing a (over) diagnose of euploid mosaic embryos in PGT-A cycles?

Summary answer: Pulling and number of laser shots employed might influence the genetic condition of the biopsied fragment leading to an over-diagnosis of normal embryos as mosaic.

What is known already: Preimplantation genetic testing for aneuploidies (PGT-A) and blastocyst biopsy is a fashion trend in assisted reproduction, but it must be performed without damaging the embryo or disrupting its genetic condition. Laser technology is commonly used to manipulate embryos in the IVF laboratory, optimizing the efficacy and reducing the exposure to suboptimal conditions.

Blastocyst stage biopsy is associated to high resolution and sensitive genetics technologies that enable mosaicism detection, increasing the reproductive chances per PGT cycle.

The main objective of this study is to find out how the biopsy technique influences the subsequent genetic results and clinical outcomes.

Study design, size, duration: Retrospective database study including 499 embryos belonging to 162 PGT-A cycles performed between August 2017 and December 2018. Cycles with semen sample concentrations under 1 million/mL were excluded. Blastocyst were biopsied by two expert embryologists using the same micromanipulation equipment. They performed two different biopsy techniques: pulling (292 embryos) and flicking (207 embryos).

Participants/materials, setting, methods: In pulling, cells were suctioned, while laser pulses were applied (4-9). In flicking, cells were aspirated and after 3-4 laser pulses they were removed by a quick movement of the biopsy needle against the holding.

Mosaic embryos with ≤25% aneuploidy were considered euploid. Euploid embryo fragments with 25-40% of aneuploidy were classified as low-grade mosaics, and those with 40-50%, were sorted as high-grade mosaics.

Student's t and Pearson's chi-square were used.

Main results and the role of chance: Pulling and flicking techniques achieved significant differences in average number of laser shots needed (6.7±2.7 vs. 3.2±0.8, respectively; p<0.0001) to obtain similar number of trophectoderm cells biopsied (5.4±1.4 vs. 5.6±1.6, respectively). No differences in amplification failure between techniques were reported (4.8% vs. 4.3%, respectively).

Similar aneuploidy rate was observed between pulling and flicking (43.0% vs. 41.3%, respectively; p=0.812) but a tendency of higher euploid mosaicism rate in the pulling group was noticed (16.1% vs. 10.2%; p=0.061). Furthermore, flicking mosaics resulted to be more complex than pulling ones: more high-grade mosaics (35.6% vs. 60.0%; p=0.066), higher number of chromosomes involved in the mosaicism (1.6±1.0 vs. 2.5±2.1; p=0.025), and higher number of segmentary alterations (1.0±1.0 vs. 1.9±2.1; p=0.029).

Until December 2018, 139 euploid single embryo transfers (SET) were performed (78 from pulling group and 61 from flicking group) and no differences in clinical pregnancy rate (34.7% vs. 32.8%) or live birth rate (32.1% vs. 27.8%) were observed, respectively. Nevertheless, it seems to be a tendency of lower implantation rate and delivery rates when flicking euploid mosaics were transferred: 45.5% implantation and live birth rate in the pulling group (11 SET) vs. 20% in the flicking group (5 SET).

Limitations, reasons for caution: This is a retrospective study, based on observation of current practice and its impact on subsequent results. Biopsy techniques were carried out by different embryologists, with the implicit bias on it. The sample size exploring reproductive outcome is small. More data must be compiled, and prospective studies carried out.

Wider implications of the findings: Even though laser used had been proved safe, more than 4 shots employed in the pulling method could modify the genetic result of the biopsy, increasing the euploid mosaicism rate.

Mosaics derived from pulling are less-complex and may implant as euploid embryos, suggesting a plausible over-diagnosis of mosaics.

Trial registration number: None

P-247 Association between IL-17A levels, oocyte fertilisation and embryo development in unexplained infertility

F. Reidy[1], R. Kavanagh[2], L. Glover[1], C. O'Farrelly[2], M. Wingfield[1]

[1]Merrion Fertility Clinic- National Maternity Hospital, Obstetrics and Gynaecology, Dublin, Ireland ;
[2]Trinity College Dublin, Trinity Biomedical Sciences Institute, Dublin, Ireland

Study question: Does dysregulation of the IL-17 pathway impact on fertilisation and embryo development?

Summary answer: Raised IL-17A levels prior to an ART cycle are associated with a reduced fertilisation rate.

What is known already: The inflammatory cytokine IL-17A plays a multifaceted role in modulating the immune response, and is thought to contribute to successful embryo implantation. We recently showed that dysregulation of the IL-17 pathway influences the outcome of ART cycles, such that elevated levels of endometrial and serum IL-17 were associated with a reduced live birth rate. In the current study, we analysed the impact of IL17 on ovarian innate immune responses, and investigated whether elevated circulating IL-17A is associated with impaired oocyte fertilisation and subsequent embryo development.

Study design, size, duration: Our aim was to investigate if serum IL-17A levels impact on embryo fertilisation and embryo development in women undergoing ART cycles. Peripheral blood samples were taken at mid-luteal cycle phase, timed with luteinising hormone testing. Serum IL-17 levels were correlated with oocyte fertilisation rates, embryo quality and pregnancy outcomes. This study also examined the effect of IL-17A, a potent proinflammatory cytokine, on the regulation of three antimicrobial peptides, Human b Defensin 1, Psoriasin and Elafin.

Participants/materials, setting, methods: 20 patients undergoing IVF/ICSI were recruited. Patients were ≤38 years, with no previous pregnancy and BMI<30kg/m². Serum IL-17A levels were measured using ELISA. An in vitro study was also performed utilizing a commercially available model of ovarian epithelia. Ovcar-3 epithelial cells were treated with recombinant human IL-17A and expression levels of the antimicrobial peptide (AMP) genes beta-defensin-1, PI3 and S100A7 were assessed by qPCR.

Main results and the role of chance: Mean fertilisation rate for oocytes retrieved from women with a serum IL-17A level of <50pg/ml (0.7283; n=9) is significantly increased compared to that of women with a serum IL17A level of >50pg/ml (0.5857; n=11); p= 0.0193. With lower circulating IL-17A there is also a trend towards improved embryo quality, but does not reach statistical significance.

IL-17A stimulation of female reproductive tract epithelial cells in vitro increased antimicrobial peptide expression. This further connects this cytokine to the mediation of the innate immune response in the ovary.

Limitations, reasons for caution: This study suggests that IL-17A can modulate innate immune responses in the female reproductive tract, including the ovarian microenvironment, in successful pregnancy. Our study is limited by the small sample size, and further study is ongoing to elaborate on these findings.

Wider implications of the findings: Our previous work has found that dysregulation of IL-17A impacts on success of ART. This further analysis suggests an effect on egg quality and subsequent embryo development. We have also shown that IL-17A induces expression of antimicrobial peptides,highlighting the role it may have in activation of the innate immune response.

Trial registration number: n/a

P-248 Soluble human leucocyte antigen-G (sHLA-G) in embryo-spent medium is a potential embryo viability biomarker and a positive predictor of live-births: a retrospective Indian study

V. Venkatappa[1], S.S. Vasan[2], S.K. Adiga[3], V. Samson Roy[4], G. Sachdeva[5], P.B. Seshagiri[6]

[1]Indian Institute of Science, Molecular Reproduction and Developmental Genetics, Bangalore, India ;
[2]Manipal Ankur Andrology & Reproductive Services-, IVF Clinic, Bangalore, India ;
[3]Kasturba Medical College-, Department of Clinical Embryology-, Manipal, India ;
[4]Advanced Fertility Clinic, IVF Clinic, Bangalore, India ;
[5]National Institute for Research in Reproductive Health-, Department of Primate Biology, Mumbai, India ;
[6]Indian Institute of Science, Department of Molecular Reproduction- Development and Genetics-, Bangalore, India

Study question: Can embryo-secreted soluble human leucocyte antigen-G (sHLA-G) serve as a predictive biomarker for embryo viability with successful pregnancy outcome?

Summary answer: Embryo-secreted sHLA-G concentrations showed positive correlations with (i) developing embryo stages and (ii) live-births. Their altered concentrations were associated with pregnancy loss or miscarriages.

What is known already: Human infertility is treated in IVF clinics with the live-birth rate being only <15%. Poor embryo viability contributes to pregnancy loss. Current embryo-grading approach has limitations in terms of identifying biologically-viable embryos that are capable of producing live-births. Several reports have indicated that sHLA-G could act as a biomarker for elective-embryo transfer, with positive IVF outcomes. But, there were controversies in terms of correlative analyses, lack of cross-clinics comparisons and to predict live-births. Hence, there is a need for a cross-clinics comparison on the embryo viability biomarker *i.e.*, sHLA-G with embryo quality assessment and correlative analysis with live-births.

Study design, size, duration: This was a three-IVF clinics retrospective study performed between Oct 2017 and May 2019, using 539 embryo-spent media (E-SMs) obtained from 539 transferred embryos from 300 sub/infertile women. Following ICSI and embryo cultures, E-SMs were stored frozen (-80° C) until subjecting them to sandwich ELISA for sHLA-G (Mybiosource, USA). Measured sHLA-G in E-SMs was correlated with embryo stages and their morphology and clinical outcomes, in terms live-births.

Participants/materials, setting, methods: We analysed E-SMs, for sHLA-G (ng/ml), from three embryonic stages *i.e.*, 4-8-cells (cleavage-stage; n = 247), morulae (n = 112) and blastocysts (n = 180). Obtained values for statistical differences were analysed between/among embryo-groups using the Student's *t-test*/ANOVA (P ≤0.05), followed by *Tukey posthoc test*. Levels of sHLA-G and embryo morphology were correlated using Pearson correlation analysis. We compared sHLA-G positive embryos with their pregnancy outcomes in terms of live-births.

Main results and the role of chance: Concentrations of sHLA-G were significantly different (P<0.05) in E-SMs of cleavage-stage (0.89 ± 0.12; n = 23) embryos *vs* blastocysts (1.81 ± 0.21; n = 11; fresh-ETs). Similarly, sHLA-G concentrations between E-SMs of cleavage-stage embryos (1.58 ± 0.17, n = 63) *vs* blastocysts (3.06 ± 0.28; n = 46; frozen-ETs) were different (P<0.05). Within and across clinic comparisons showed that concentrations of sHLA-G in E-SMs of cleavage-stage embryos, morulae and blastocysts were variable. A significant difference (P<0.001) was observed in sHLA-G concentrations only with cleavage-stage embryos but not with morulae or blastocysts, indicating embryo development stage-wise correlations, albeit, with variability within and across clinics. Furthermore, there was a moderate relationship between cleavage-stage embryo morphology and sHLA-G levels and, a positive correlation of the blastocyst grades with sHLA-G concentrations. Interestingly, sHLA-G levels were higher in live-births cases *vis-à-vis* no-birth cases; the sHLA-G concentration was higher in live-births out of blastocyst-ETs (1.79 ± 0.28, n = 22) *vs* those out of cleavage-ETs (1.44 ± 0.24; n = 42). Live-births were observed only when at least one sHLA-G positive embryo was a part in twin- or triple- ETs. These results show a clear association of embryo-secreted sHLA-G levels with pregnancy outcome with live-births.

Limitations, reasons for caution: Variations of sHLA-G contents in E-SMs between different IVF clinics could be attributed to protocol-practices differences of clinics. More multi-centric larger-cohort studies and a large scale meta-analysis are required to establish the diagnostic accuracy power of the sHLA-G for predicting the IVF outcomes in term of child-birth.

Wider implications of the findings: For the first time, our multi-centric Indian study, show that the sHLA-G could be an embryo viability biomarker to predict child-birth. The sHLA-G could potentially be an immunodiagnostic point-of-care diagnostic test, a valuable adjunct to other criteria practiced for selection of potentially 'top-quality' embryo(s) for ET.

Grant: MHRD-ICMR, India.

Trial registration number: not applicable

P-249 The removal of a quarter of the Zona Pellucida improves pregnancy and implantation rates in vitrified blastocysts that underwent Artificial Shrinkage

A. Volpes[1], F. Sammartano[1], M. Modica[1], S. Gullo[2], A. Marino[1], A. Allegra[1]

[1]ANDROS Day Surgery Clinic, Reproductive Medicine Unit, Palermo, Italy ;
[2]University of Palermo, Department of Psychology- Educational Science and Human Movement- Statistics Unit-, Palermo, Italy

Study question: Is the partial removal of the Zona Pellucida (ZP) effective in improving pregnancy and implantation rates in vitrified blastocysts undergoing Artificial Shrinkage (AS)?

Summary answer: Partial removal of the ZP significantly improves pregnancy and implantation rates in blastocysts that underwent AS either before vitrification or after thawing.

What is known already: The hatching process is an essential mechanism enabling blastocyst implantation. It is well known that vitrification may cause hardening of the ZP and this event could explain implantation failure after blastocyst thawing and transfer.

However, due to the thinness of the ZP in the expanded blastocysts, Laser Assisted Hatching (LAH) is almost impossible to perform.

Moreover, published articles state that the blastocoelic fluid may cause inadequate vitrification resulting in lower pregnancy and implantation rates.

To overcome this problem, a reduction in the volume of the blastocyst has been proposed, by using pre-vitrification AS to allow the blastocoelic fluid leak.
Study design, size, duration: Retrospective observational study. All procedures were performed at Andros Day Surgery Clinic, Reproductive Medicine Unit, Palermo, Italy.

We included 409 warming cycles (January 2017-October 2019) (625 blastocysts; 397 embryo transfer).
Participants/materials, setting, methods: We compared outcomes in cycles with blastocysts cryopreserved performing AS and LAH (group A) and blastocysts transferred without performing AS nor LAH (group B).

Moreover, group A was divided in A1 (where AS was performed before vitrification; after thawing, LAH was applied to partially remove ZP) and A2 (where blastocysts were vitrified without AS; after thawing, in the expanded blastocysts, AS and LAH were performed; if blastocysts were still spontaneously collapsed, only LAH was conducted).
Main results and the role of chance: Significant differences were determined using chi-square with Yates' correction and Fisher's exact tests. Independent t-test was computed for continuous variable (age). A P-value < 0.05 was considered significant.

Group A included 248 blastocysts transferred in 162 cycles. Group B included 345 blastocysts transferred in 235 cycles.

There is no significant difference in maternal age between two groups (33.1±5.4 vs 32.05±5.5 p=0.06).

Survival rate is the same in both groups (group A: 96.9% and group B: 93.5% p=0.07).

Both pregnancy and implantation rates are significantly higher in group A than group B (pregnancy rate=47.5% vs 35.3% p=0.019; implantation rate=38.3% vs 28.7% p=0.018).

No differences are shown regarding multiple pregnancy rates (22.1% vs 19.3% p=0.81) or miscarriage rates (26.0% vs 25.3% p=0.93).

We also compared group A1 and group A2.

Group A1 included 200 blastocysts transferred in 127 cycles.

Group A2 included 48 blastocysts transferred in 35 cycles.

Survival (97.6% vs 94.1% p=0.20), pregnancy (48.8% vs 42.9% p=0.53), implantation (38,0% vs 39.6% p=0.84), multiple pregnancies (26.7% vs 21,0% p=0.89) and miscarriage (24.2% vs 33.3% p=0.69) rates are not statistically different.
Limitations, reasons for caution: The study, being retrospective, limits its value. Furthermore, group A2 is less numerous than the others.
Wider implications of the findings: Our results seem to indicate that the increased outcomes are not correlated to the AS. The removal of a quarter of the ZP before embryo transfer (as in blastocysts collapsed before vitrification and blastocysts collapsed after thawing) could be the key to the improved outcomes.
Trial registration number: Non applicable

P-250 Maternal age alters morphokinetics of embryos competent to provide a live birth

M.C. Guglielmo[1], A. Bartolacci[1], E. De Ponti[2], F. Brambillasca[1], M. Mignini Renzini[1], J. Buratini[1,3], D.C. Mariabeatrice[1]

[1]Biogenesi - Reproductive Medicine Centre, Istituti Clinici Zucchi, Monza, Italy ; [2]ASST Monza, Dept Medical Physics, Monza, Italy ; [3]Sao Paulo University - Institute of Biosciences, Department of Physiology, Botucatu, Brazil

Study question: Do morphokinetic parameters until the eight-cells stage differ between embryos competent and noncompetent to provide a live birth, and are they influenced by maternal age?
Summary answer: Embryos competent to provide a live birth display faster early developmental kinetics than those noncompetent. Maternal age reduces kinetic differences between competent and noncompetent embryos.
What is known already: Time-lapse microscopy (TLM) has been increasingly applied to improve accuracy in embryo selection. However, the lack of studies assessing the association of morphokinetic markers in embryos with their competence to provide a live birth (LB) represents a significant knowledge gap. The performance of TLM-based algorithms has varied among different clinical settings, and patient profile, particularly maternal age, has been suggested as a significant driver of this variation. Nevertheless, to our knowledge, no study so far has assessed the impact of maternal age on the morphokinetics of embryos competent to provide a LB.
Study design, size, duration: Retrospective study including 816 ICSI first cycles, from 2014 to 2018. 4915 embryos were produced, from which 2093 were morphologically selected to be transferred in SET or DET 3 days after ICSI, resulting in 168 LB and 1176 non-implanted (NI) embryos with trackable morphokinetics. Morphokinetic parameters were compared between LB and NI embryos, and within each embryo type, between patients <37 or ≥37 years. The correlation between morphokinetic parameters and LB rates was tested.
Participants/materials, setting, methods: After ICSI, embryo culture was performed in a TLM incubator. The morphokinetic parameters assessed were tPNf, t2, t3, t4, t5 and t8, which were compared between groups with different embryo performance (LB *vs.* NI), and between LB embryos from patients < 37 and ≥ 37 years by Wilcoxon test. The association between morphokinetic parameters and LB rates was assessed by multivariate regression analysis including maternal age and BMI as continuous variables in the model.
Main results and the role of chance: All morphokinetic parameters were reached significantly earlier by LB embryos compared to NI embryos [means(hours)±SD; tPNf: 22.5±2.7 *vs.* 24±3.4; t2: 25±2.7 *vs.* 26.8±3.7; t3: 36.1±3.3 *vs.* 37.7±4.5; t4: 36.8±3.6 *vs.* 39.1±5.1; t5: 48.8±4.9 *vs.* 50.1±6.4; t8: 52.7±6.1 *vs.* 55.1±6.6; (p<0.0001)]. LB embryos from patients < 37 years reached all morphokinetic endpoints faster than LB embryos from patients ≥ 37 years [tPNf: 22.3±2.7 *vs.* 23.2±2.4; t2: 24.8±2.8 *vs.* 25.7±2.5; t3: 35.7±3.1 *vs.* 37.4±3.4; t4: 36.4±3.5 *vs.* 37.9±3.6; t5: 48.1±4.7 *vs.* 50.8±5; t8: 52.2±6 *vs.* 54.2±6.2; (p<0.05)]. There were no differences between morphokinetic parameters from NI embryos obtained from patients < 37 and ≥ 37 years. Consequently, differences between LB and NI morphokinetics were more evident in patients < 37 years, compared to those ≥ 37 years. Multivariate regression analysis revealed that, independently of maternal age and BMI, tPNf, t2, t5 and t8 are significantly associated with LB rate in the overall patient population [tPNf: 0.7 OR, (0.4-1.0) 95% IC, p=0.048; t2: 0.7 OR, (0.4-1.0) 95% IC, p=0.048; t5: 1.1 (0.6-1.0), p=0.01; t8: 0.9 (0.9-1.0, p=0.047)].
Limitations, reasons for caution: Our study is subjected to the intrinsic limitations of a retrospective analysis, the results presented could have been affected by variables that are uncontrolled for.
Wider implications of the findings: The present data indicate that early morphokinetics can be used to predict embryo developmental competence, but its predictive power decreases with maternal age. Therefore, our study provides valuable references for the design and application of embryo selection strategies utilizing TLM-derived information.
Trial registration number: Not Applicable.

P-251 How many donor eggs are needed in an IVF-DO cycle?

B. Marqués López-Teijón[1], A. Garcia-Faura[1], S. Novo[1], F. Garcia[1], C. Castello[1], M. Lopez-Teijon[1]

[1]Institut Marques, Reproductive Medicine Service, Barcelona, Spain

Study question: Is the largest number of donor eggs always associated with better results?
Summary answer: Good results can be obtained with an adjusted number of mature oocytes and a greatly increasing the number of ovules provides a limited value.
What is known already: There is much literature on the number of eggs necessary to obtain success in an IVF of their own, but scarce about how many mature eggs are necessary for an optimal result in case of IVF with donor eggs. Fertility clinics suffer the pressure of patients who need donor eggs because they believe that the greater the number, the more likely they are to succeed.
Study design, size, duration: We have conducted a single center retrospective observational study. The result of IVF cycles with egg donation is compared according to the number of mature oocytes assigned. 1,648 consecutive IVF with donor eggs cycles were analyzed over a period of two years (2017-2019). Three groups were created according to the number of mature oocytes assigned: A. 4 mature oocytes in metaphase II (396 cycles), B. 5-7 oocytes (620 cycles) and C. 8-10 oocytes (632 cycles).
Participants/materials, setting, methods: The groups are comparable in mean age of donors (26.2 years) and recipients (43.0 years), in percentage of fresh (91-94%) and vitrified (6-9%) oocytes, and in percentage of fresh embryo

transfers (35-37%) and frozen (63-65%). All embryo transfers were of single blastocyst of superior or equal quality to 3BB (Gardner).
Main results and the role of chance: Statistically significant differences ($p < 0.05$) have been found in the average of good quality blastocysts obtained per cycle: 2.1 in group A, compared to 3.1 and 3.2 in groups B and C. There are also statistically significant differences in the pregnancy rate per cycle: 85% in group A, compared to 91% and 90% in groups B and C. No statistically significant differences were found between the groups in the pregnancy rate per transfer (61,5% to 65,2%) or in the newborn rate per transfer (44,2% to 47,2%).
Limitations, reasons for caution: It's a retrospective single center study, these results will vary depending on the success rates of each laboratory.
Wider implications of the findings: Increasing the number of eggs up to a 250%, the pregnancy rate per cycle rises just a 5%. These data suggest that good results can be obtained with an adjusted number of mature oocytes and that greatly increasing the number of eggs provides limited value.
Trial registration number: not applicable

P-252 Reproductive potential of conventional IVF and ICSI on sibling oocytes in the case of isolated teratozoospermia.

N. Thondehal Math[1], G. Devika[1], G. Deepanjali[1], R. Sajana[1], R. Anjana[1], B. Vidhya[1], W.Y. Son[2]

[1]Gunasheela Surgical & Maternity Hospital, embryology, Bangalore, India ; [2]McGill University Health Centre, Embryology, Montreal, Canada

Study question: Does ICSI have any embryological and clinical benefits for the couple who have isolated teratozoospermia compared to conventional IVF insemination?
Summary answer: Reproductive potential is similar between both insemination methods and so, couples with isolated teratozoospermia need not be subjected to ICSI.
What is known already: In many studies, semen samples with teratozoospermia produced lower fertilization rates when conventional IVF was used. On the other hand, in some studies, patients with teratozoospermia achieved good fertilization in conventional IVF as long as the sperm concentration and motility were within the normal range (isolated teratozoospermia) according to WHO standards. Few studies observed the difference in embryo quality and further clinical outcomes of patients with isolated teratozoospermia between conventional IVF and ICSI. This study compares reproductive potential between conventional IVF and ICSI from the same patients with isolated teratozoospermia.
Study design, size, duration: These prospective studies were conducted in a private hospital between April 2015 and December 2019. In each case, sibling oocyte cumulus complexes were randomized to be inseminated either by ICSI (n=1555) or IVF (n=471) (4: 1 ratio) to prevent completely failed fertilization. Fertilization, good blastocyst rates (≥ 4BB) and clinical outcomes in ICSI group were compared to IVF group.
Participants/materials, setting, methods: This study was conducted in patients (n= 102, age= 34.7 ± 4.6 years old) for whom at least 10 oocytes were retrieved and partner had isolated teratozoospermia. In the ICSI group, 1159 oocytes out of total 1555 oocytes were mature and underwent ICSI, and the rest underwent conventional IVF. All fertilized embryos were cultured to blastocyst stage. The best blastocysts produced were transferred in either fresh or frozen cycles.
Main results and the role of chance: Fertilization and blastocyst utilization rates were similar between ICSI group (75.8%, 878/1159; 67.8%, 595/878) and IVF group (74.5%, 351/471; 71.5%, 251/351) ($P > 0.05$). Failure of fertilization after IVF insemination occurred in 5 of the 102 couples (4.9%) and in none with ICSI. After transferring the embryos either in fresh or frozen cycles (n=94) there was no statistically significant difference in clinical pregnancy and ongoing/live birth rates among cycles transferred from ICSI (58.13%, 25/43; 53.4%, 23/43), IVF (64.7%, 22/34; 47.1%, 16/34) and combined groups (61.7%, 11/17; 58.8%).
Limitations, reasons for caution: The sample size is low. Although number of sibling oocytes should have been put into IVF group, the design was intended to prevent total fertilization failure.
Wider implications of the findings: Since failed fertilization rate in IVF insemination on normospermic infertility factor patients is also over 5%, our study implies that IVF insemination is an option to avoid unnecessary use of ICSI which is time-consuming, costly and has potential risks for the couples with isolated teratozoospermia.
Trial registration number: not applicable

P-253 Oocyte incubation time prior to ICSI and its impact on assisted reproduction results. A randomized controlled trial

R. Pesce[1], A. Piazza[1], R. Rolando[1], C. Elizondo[2], G. Perman[3]

[1]Hospital Italiano de Buenos Aires, Gyneacologucal Department, Buenos Aires, Argentina ; [2]Hospital Italiano de Buenos Aires, Epidemiology Department, Buenos Aires, Argentina ; [3]Hospital Italiano de Buenos Aires, Public Health Department, Buenos Aires, Argentina

Study question: Are there differences in oocyte maturity, fertilization rate and early embryo evolution (cleaving embryo) between early and late oocyte denudation immediately prior to ICSI?
Summary answer: Early oocyte denudation is better than late denudation in percentage of: MII oocytes (OR 2.38; p 0.013) and cleaving embryos (OR 2.30; p 0.028).
What is known already: The time of contact between cumulus cells and the oocyte play an important role in the achievement of oocyte competence, oocyte aging process and early embryo development. In addition, the time from trigger administration to retrieval, and the time in culture before insemination affect oocyte maturation and ovarian ageing in vitro.

There is controversy in international scientific literature regarding oocyte incubation time. Studies thus far were done in women with fertility issues, or in donor women but with retrospective designs.

The best timing of incubation prior to ICSI is yet to be determined.
Study design, size, duration: Design: randomized controlled trial. Oocytes were randomized to early or late denudation time (two arms). They were the unit of analysis. Since oocytes coming from each donor woman were considered to have a cluster effect, we adjusted for this with a random-effects multilevel model.

Sample size: 184 oocytes per treatment arm (accounting for the design effect) to have 80% power and 5% alpha error to detect a 20% difference in results.

Duration: January 2016-December 2018.
Participants/materials, setting, methods: Setting: Oocyte donation programme in a university hospital.

Participants: Randomized oocytes came from healthy women (donors).
Methods: Treatment arms: Early denudation: thirty minutes after oocyte pick-up (OPU), oocytes were denudated from its cumulus. Late denudation: four hours later than OPU. All oocytes were subject to ICSI immediately after denudation.

All participants, professionals and researchers were blinded to treatment allocation except for the biologist that performed the process (who did not participate in other activities).
Main results and the role of chance: Out of a total of 375 included oocytes, 191 were randomly assigned to early denudation (ED) arm, and 184 to late denudation (LD). The mean time to denudation was: ED, 0.5 hours (95% CI 0.42 – 0.58); LD, 4 hours (4 – 4.5); p <0.001.

The crude percentage of MII oocytes was 91.6% and 83.7% (ED vs LD, respectively), p 0.033. The crude percentage of fertilized oocytes was 55.3% vs 44.7%, p 0.642. The crude percentage of cleaving embryos was 56.9% vs 43.1%, p 0.012.

The adjusted odds ratio (OR) for MII oocytes (comparing early vs late denudation) was 2.38 (95% CI 1.20 – 4.72); p 0.013. The adjusted OR for fertilization was 1.30 (0.68 – 2.48); 0.423. The adjusted OR for cleaving embryo was 2.30 (1.10 – 4.85); p 0.028**Limitations, reasons for caution:** This is the first randomized controlled trial that evaluates early vs late denudation in oocytes from healthy women. Its results can encourage the production of new studies that confirm findings and assess its potential impact in clinical outcomes (pregnancy rate and live birth rate).
Wider implications of the findings: These findings suggest that early denudation could improve the odds of having cleaving embryos at third day after ICSI. This could mean a need for change in usual embryology laboratory processes.
Trial registration number: ClinicalTrials.gov Identifier: NCT03121924

P-254 Make hey while sun shines! Hormone 25 OH D (vitamin D3) in follicular fluid: a determinant factor for top grade blastocyst formation?

N.M. Chimote[1], B. Chimote[2]

[1]Vaunshdhara Fertility Centre, Embryology & Reproductive Endocrinology, Nagpur, India ; [2]Vaunshdhara Fertility Centre, Embryology- Endocrinology, Nagpur, India

Study question: To investigate relationship of 25(OH) D (vitamin D3) in follicular fluid (FF) with oocyte competence to fertilize, cleave and for top quality blastocysts.
Summary answer: Follicular-fluid level of 25(OH)D (Vitamin D3) is a potentially predictive marker for oocyte competence to fertilize, cleave and form top-grade blastocysts in women undergoing IVF.
What is known already: There is growing evidence that [25(OH)D has very important role in human reproduction. Vitamin D3 contributes to restoration of the menstrual cycle and endometrial proliferation, growth of follicles, improves primary dysmenorrhea, and reduce occurrence of uterine fibroids. However, owing to conflicting results, the relationship of serum levels of Vitamin D3 with ovarian stimulation characteristics or with embryo quality has been rather obscure. IVF provides a unique opportunity to explore such a relationship as measurement of vitamin D3 in follicular-fluid can help trace the fate of individual oocytes, their fertilization and embryonic development.
Study design, size, duration: This non-randomized prospective study included women (n=300, 22-42 years) undergoing IVF during January2017-December2019. None of the patients received vitamin D3 supplementation before Controlled Ovarian Hyperstimulation (COH). Follicular-fluid collected from first aspirate of individual follicle was pooled, for each patient, to measure Vitamin D3 levels using RIA kits. Embryonic development from fertilization to blastocyst formation was recorded. Embryo gradation was done as per conventional criteria. All blastocysts were vitrified for next natural cycle embryo transfer.
Participants/materials, setting, methods: Women with endometriosis, tuberculosis and hydrosalpynx and their male partners with severe or moderate male factor were excluded from this study. Women with Poor ovarian response (≤ 3 retrieved oocytes) to recombinat FSH / gonadotropin stimulation were also excluded. FF Vitamin D3 levels were divided into Low and High groups as per their median value. Fertilization, cleavage and blastocyst formation rates were recorded in low and high FF vitamin D3 groups.
Main results and the role of chance: Age, number of eggs retrieved and MII oocytes were comparable between Low FF Vitamin D3 Group (< 41 ng/ml, n=152) and High FF Vitamin D3 groups (> 41 ng/ml, n= 148). % fertilization, % cleavage was significantly higher in low group than high vit D3 group (93.3 ± 22.6 vs 86.1 ± 29.5% P: 0.0205). Overall Blastocyst formation remained similar (50.3 ±37.9 vs. 54.1 ± 35.4% : P value 0.3740). However, top and good grade blastocysts were significantly higher in low FF/vit. D3 group than in high FF/vit. D3 group (Top: 34.4 ±0.4 vs 24.2 ±0.8%, P= 0.0183 and Good: 37.8 ± 0.69 vs 21.4 ± 0.72, p= 0.0002).

Interestingly, poor quality blastocysts were significantly high in high FF/ vit. D3 compared to FF/low vit. D3 group (45.0 ± 38.5 vs 26.3 ± 33.0 %, P value: < 0.0001). Thus, measurement of vit. D3 in follicular fluid has tremendous potential to identify the embryonic development to blastocyst stage with top or good quality so as to select the best embryo for transfer. It also helps enhance the chances of getting a viable pregnancy resulting in live birth.
Limitations, reasons for caution: Since this study has very limited number of cases, more multicentric studies should be carried out to endorse impact of vit. D3 on embryonic development. Hence, pre treatment of supplementation of Vit D3 also should be contemplated according to variability of sunlight all over the world.
Wider implications of the findings: With changes in weather and carbon emission with problems in ozone layers all over the world, it seems that natural synthesis of vit. D3 is disturbed. Hence, proper evaluation of this hormone should be judiciously done to improve excellent embryo development and a viable pregnancy.
Trial registration number: not applicable

P-255 A freeze-all strategy in a donor egg program

A.M. Quinteiro Retamar[1], M. Miguens[2], A. Coscia[2], N. Allende[2], G. Vega Balbuena[2], D. Acosta[2], G. Fiszbajn[1]

[1]CEGYR, EGG DONATION, caba, Argentina ;
[2]CEGYR, clinical, caba, Argentina

Study question: To compare ongoing pregnancy and miscarriage rate after blastocyst transfer in an oocyte donation program in patients with synchronization of donor-recipient cycle versus freeze-thawed strategy.
Summary answer: A freeze-all strategy may be an advantage allowing to schedule treatments without cancellation or delays, but the reproductive results have not shown statistical difference.
What is known already: Theoretically, freeze-thawed embryo-transfer (ET) may have better ongoing pregnancy rate than fresh ET due to a more favorable intrauterine environment by avoiding the condition after ovarian stimulation. It is although thought that the pregnancy potential of blastocyst frozen on day 5 and slow developing day 6 blastocysts, after a freeze-thawed strategy, are comparable. Synchronization of the donor-recipient cycle may have some difficulties such as cancellation of the treatment, either of the donor stimulation or the recipient endometrial preparation or the possibility of the absence of blastocyst. These challenges may disappear with a freeze-all strategy.
Study design, size, duration: This was a retrospective cohort study conducted at CEGyR, Buenos Aires, Argentina. A total of 650 women who were recipients having her first single embryo transfer from March 2016 and November 2019 were included.
Participants/materials, setting, methods: A total of 650 recipients between 28-51 years who were having her first single ET were included. All ET were performed at blastocyst stage. The patients were separated in two groups: (1) ET was made with a synchronization of donor-recipient cycle (2) ET was made after a freeze-all strategy. The analyzed variables were ongoing pregnancy rate and miscarriage rate by chi square test. (The ongoing pregnancy rate determined by transvaginal ultrasonography at gestational weeks 8-10).
Main results and the role of chance: A total of 392 patients were included in group 1 and 258 patients in group 2. The average age in group one was 41.5 years old and in group two 42.1 years old. In the former group the ongoing pregnancy rate was 47,70% (n=187) vs 43,41% (n=112) in the latter group (IC95 0,76-1,08 p=0.14). The miscarriage rate in the first group was 11,48% (n=45) vs 14,34% (n=37) in the second group (IC95 0,86-1,86 p=0.11). Both groups have similar results and they were not statistical significance.
Limitations, reasons for caution: The main limitation of this study was its retrospective design based on data from a single center which may be subject of bias.
Wider implications of the findings: While the freeze-all strategy is a more practical way to schedule treatments, it has not shown better clinical results. However, prospective randomized studies are needed to confirm the hypothesis and a cost-effectiveness analysis to determine its applicability.
Trial registration number: NOT APPLICABLE

P-256 The association of early-cleavage embryo morphokinetics and blastocyst formation rate does not vary by women age: a time-lapse monitoring study

A. Gomes[1], H. De Martin[1,2], M. Fujji[1], M. Conatti[1], M. Nakano[2,3], T. Bonetti[4,5], P. Monteleone[2,6]

[1]Monteleone - Human reproduction center, Embryology, São Paulo, Brazil ;
[2]Universidade de São Paulo - USP, Gynecology Discipline - Obstetrics and Gynecology Department, São Paulo, Brazil ;
[3]Monteleone - Human reproduction center, Clinical, São Paulo, Brazil ;
[4]Monteleone - Human reproduction center, Scientific, São Paulo, Brazil ;
[5]Universidade Federal de São Paulo, Gynecology Department, São Paulo, Brazil ;
[6]Monteleone - Human reproduction center, Clinical director, São Paulo, Brazil

Study question: Does the embryo morphokinetics, from early cleavage until blastocyst formation based on time-lapse monitoring (TLM), vary according to women age?
Summary answer: The time-range of embryos progressing from two to three-cell (CC2) is associated to blastocyst formation and does not vary by women age
What is known already: ART success depends on a number of factors and one of the most significant is the women age. Reproductive capacity in women declines with ageing, associated with a decrease in oocyte quantity and quality, which correlates with lower fertilization rates and poorer embryo development. Embryo selection is a crucial step in ART and the introduction of TLM provided additional noninvasive criteria for better embryo selection and embryonic kinetics markers starting from the first cleavage. Early cleavage has been described as a key factor for embryo selection, however, there is studies evaluating the variation in these parameters according to maternal age.
Study design, size, duration: This retrospective cohort study used prospectively collected data from 671 ICSI cycles from 486 patients between April/2018 and September/2019 in a private reproductive medicine center. A total of 4724 mature (MII) oocytes were fertilized by ICSI and cultured in a time-lapse monitoring system (Embryoscope®). The time-lapse monitoring parameters and

blastocyst formation rate (BlastR) were compared in groups according to women age (<38 years or ≥38 years).

Participants/materials, setting, methods: Early-cleavage parameters were analyzed and correlated to blastocyst formation in each group. The time between two-cell and three-cell (cell cycle two – CC2), three-cell and five-cell (CC3), five-cell and nine-cell (CC4) were calculated. The blastocysts were classified according to morphology by Gardner's classification and those with trophoectoderm grade ≥3 and inner-cell mass A or B were considered top-quality. The blastocyst rate (BlastR) was calculated by dividing the number of blastocysts obtained by number of 2PN.

Main results and the role of chance: From the 4724 MII oocytes injected, 3622 were normally fertilized (2PN). From those, 1635 came from women <38 years (48.8%) and 1716 from women ≥38 years (51.2%). 271 2PN oocytes were excluded from analysis due to missing data. The mean time of CC2 (<38-years: 10.0±4.7 vs ≥38-years: 10.5±4.8; p=0.005), CC3 (<38-years: 12.4±6.6 vs ≥38-years: 12.7±6.8; p=0.212) and CC4 (<38-years: 23.1±8.9 vs ≥38-years: 23.4±9.0; p=0.300) did not clinically differed between age groups. A total of 2159 embryos developed to blastocyst stage (64.5%) and the BlastR did not clinically differed between <38-years group (66.4%) and >=38-years group (62.6%, p=0.023) as well. The BlastR varied according to CC2 and the higher BlastR occurred when embryos presented CC2 between 8 and 13 hours for both groups (<38-years: 80.5% vs ≥38-years: 75.9%; p=0.007). For the top-quality blastocyst rate (TQ-BlastR) the same pattern was observed and TQ-BlastR when CC2 was between 8 and 13 hours were (<38-years: 59.9% vs ≥38-years: 49.5%; p<0.001). Despite of statistically significance observed in some parameters compared between groups <38-years vs ≥38-years, those did not have clinical impact as the means are very close and the statistical differences occurred possibly by the high number of embryos included in the analysis.

Limitations, reasons for caution: This retrospective study evaluated the association of CC2 and blastocyst formation according to women ages. The blastocyst choosing criteria was based on blastocyst morphology and did not take CC2 into account. Then, we could not evaluate the impact of CC2 on pregnancy success.

Wider implications of the findings: We can underline that time of CC2 predict blastocyst formation and it does not vary according to women age. Although the pregnancy rate decreases in older women, our findings support the hypothesis that the morphokinetics of embryos from the first cleavage to the blastocyst formation does not vary with age.

Trial registration number: not applicable

P-257 Two Labs-Two countries: five-years' report with cumulative live birth rate of the ovum donation program in an Italian University Hospital

R. Picone[1], F. Bertocci[1], R. Fucci[1], P. Falcone[1], C. Giachini[1], E. Borrani[1], M.E. Coccia[1], G.M. Cito[1], L. Badolato[1], P. Evangelisti[1], F. Rizzello[1], D. De Angelis[1], C. Cozzi[1]

[1]Careggi University Hospital, assisted reproduction technology, Florence, Italy

Study question: Two labs-two countries' for the egg donation program: how is it efficient in terms of cumulative life birth rate (CLBR)?

Summary answer: Combining fresh/frozen embryo transfer in the same couple, deriving from donor vitrified and travelling oocytes is an efficient way to improve significantly the CLBR.

What is known already: Vitrification of oocytes and embryos is the golden standard used worldwide for the cryopreservation in IVF and fertility preservation programs. The IVF outcome, in terms of pregnancy and implantation rate, are comparable with those obtained with fresh oocytes/embryos. Advances in reproductive techniques, i.e. the introduction of oocytes vitrification, has allowed simplifying the donation process by the formation of egg banks. The extremely low rate of egg donation by Italian women does not satisfy the current level of demand and over 90% of the IVF cycles with egg donation originated from foreign (mainly Spanish) gamete bank.

Study design, size, duration: Retro-prospective study on our couples from 2015 to 2019. In our five-years' experience we have been used the so-called 'two country-two lab' model meaning that the oocytes retrieval and the subsequent vitrification is performed in foreign banks while their fertilization after warming by ICSI and embryo transfer is made in our laboratory. Thanks to collaboration between our laboratory and the foreign banks we were able to optimize the entire procedure.

Participants/materials, setting, methods: For each egg donation cycle we receive six oocytes. The kitazato protocol was utilized for oocytes warming. Banks can be asked to send 3 more oocytes if the survival rate was less than 50%. Rates of oocytes survival, fertilization, cleavage, clinical pregnancy and life birth were reported, including CLBR. Embryo transfer and cryopreservation were performed on day 3 or 5.

Main results and the role of chance: From 2015 to 2019 a total of 900 egg donation cycles were performed. The oocyte survival rate is 81,0±19,3%. The fertilization rate is 70,0±25%. The cleavage rate is 91,0±14,2%. The percentage of pregnancy rate in the fresh transfer is 36% with a life birth rate of 26%.

30.5% of patients underwent ET also have cryopreserved embryos, thus they have the chance to have subsequent ETs.

Considering the cumulative percentage, i.e. including ET with warmed embryos, the pregnancy and the life birth rates increase significantly, up to 40% and 32% respectively (p <0.05 for both parameters).

These results indicate that cryopreserved embryo transfers contribute substantially to the total success rate of an IVF cycle with egg donation.

Limitations, reasons for caution: Cumulative life birth rate has been calculated on concluded IVF cycles, i.e. if life birth was achieved or when no more vitrified embryos were available

Wider implications of the findings: Our findings confirmed the efficacy of the double vitrification-warming procedure (at oocytes and embryos stage) that could be used for supernumerary embryos or to postpone embryo transfer when the endometrial pattern is suboptimal.

Trial registration number: not applicable

P-258 Evaluation of the relation between the value of mitochondrial DNA (MitoScore) and euploid embryo quality

M. Niag dos Santos Rocha[1], D. Freitas[2], R. Carvalho[3], T. Oliveira[3], C. Santos[3], T. Abreu[3], C. Teixeira[2], A. Almeida[2], L. Queiroz[2], G. Coelho[4]

[1]Universidade Federal do Recôncavo da Bahia, Centro de Ciências da Saúde, Santo Antônio de Jesus, Brazil ;
[2]Insituto Valenciano de Infertilidade - IVI Salvador, IVF Lab, Salvador, Brazil ;
[3]Universidade Federal do Recôncavo da Bahia, Medical School, Santo Antônio de Jesus, Brazil ;
[4]Insituto Valenciano de Infertilidade - IVI Salvador, Clinical Director, Salvador, Brazil

Study question: Can the copy number of Mitochondrial DNA (mDNA) – MitoScore – be associated with quality of an euploid embryo?

Summary answer: The evaluation of the number of copies of mitochondral DNA (Mitoscore) does not correlate with the quality of the embryo.

What is known already: Assisted reproduction has tried to identify and develop strategies that make it possible to increase the rates of embryonic implantation and, consequently, pregnancy. Implantation failure rates with morphologically and chromosomally normal embryos show that there are other factors that affect embryonic quality and fertilization rate. Mitochondria and their DNA (mDNA) have been suggested as a potential marker of embryonic viability and chances of fertilization, since their copies number is related to the energy supply of the embryo. It is known that a larger number of copies of mDNA in a blastocyst seem to be associated with a lower implantation rate.

Study design, size, duration: This is a descriptive retrospective, observational, cross-sectional study. A convenience sample was used, covering the analysis of medical records of couples diagnosed with infertility, who underwent treatment at an assisted reproduction clinic in Salvador, Bahia, Brazil, in total convenience sample of 69 cycles, from a total of 69 patients. This work was developed during the period from from August 2016 to December 2019.

Participants/materials, setting, methods: This study evaluated 173 embryos which underwent preimplantation genetic testing for aneuploidy (PGT-A) and MitoScore testing (on euploid embryos) was performed by Igenomix. Embryos were classified on the basis of embryo quality (EQ) utilizing ASEBIR criteria into three groups: Good (AA/AB/BA/BB), Fair (BC/CB), Poor (CC). Software Statistical analysis was used and the differences between three groups was estimated by ANOVA test. In all cases, p < 0.05 was considered statistically significant.

Main results and the role of chance: Data from a total of 69 cycles, of 69 women, With an average age of 36.31 (+ 5.29) years. Of the 173 embryos analyzed, there were 115 (66.47%) embryos with Good EQ, 41 (23.70%) embryos with Fair EQ and 17 (9.83%) embryos with Poor EQ. In embryo group with Good EQ, the average of Mitoscore value was 23,29 (+ 5.98); in group with Fair EQ, the average of MitoScore value was 22,76 (+ 5.42); and in group with Poor EQ, the average of MitoScore value was 24,12 (+ 6.07). There was no statistically significant difference between the groups evaluated (*p=0.7167*). In addition, it was also observed that of the 173 embryos analyzed, 86 were transferred, in a total of 63 transfer procedures (with one or two embryos). Of this total transfer, 29 procedures were implanted, which represents a 46.0% implantation rate.
Limitations, reasons for caution: The reduced number of embryos analyzed in this study does not allow establishing more precise correlations between analyzed variables. This study will be complemented with new cycles to increase the investigation about importance of MitoScore in prediction of embryo viability and implantation rates.
Wider implications of the findings: There is no association between embryo quality (EQ) and embryo's MitoScore in this study. This result is in agreement with evidences in literature. However, the knowledge of this value can be useful in the decision in order of embryonic transfer, when having embryos with similar EQ.
Trial registration number: Not applicable

P-259 Effect of Myo-Inositol and Alpha-Lipoic Acid on oocyte morphology and embryo morphokinetics: a prospective preliminary analysis of 40 overweight patients undergoing ICSI treatment

G. Gennarelli[1], C. Paschero[2], S. Canosa[1], C. Benedetto[1], A. Revelli[1]

[1]Turin University, Obstetrics & Gynaecology, Torino, Italy ;
[2]Turin University, Obstetrics & Gynecology, Torino, Italy

Study question: May the administration of a multivitamin compound based on Myo-Inositol, Alpha-Lipoic Acid and Folic Acid before controlled ovarian stimulation influence oocyte and embryo quality?
Summary answer: The administration of Myo-Inositol, Alpha-Lipoic Acid and Folic Acid may affect both oocyte and embryo morphological features and reproductive outcomes in overweight patients
What is known already: Several clinical studies have demonstrated that both Myo-Inositol and α–Lipoic Acid are involved in mitochondrial respiratory chain and energy metabolism having a positive effect oocyte quality and embryo development of women affected by PCOS. Therefore the administration in PCOS patients may improve reproductive outcomes by both affecting metabolic pathways and reducing oxidative stress. Nevertheless, data are missing on the use of Myo-Inositol in women defined poor responders undergoing ovarian stimulation for ICSI cycle. Therefore, the aim of the present study was to provide evidence on the effect of this compound in overweight patients
Study design, size, duration: This is a randomized prospective analysis of 40 patients aged 25-40 years, with 27≤BMI≤32, AMH≥0.1, FSH≤15 without diagnosis of diabetes and autoimmune diseases undergoing ICSI treatment at our IVF Unit between 2018 and 2019. According to the randomization patients assumed either the multivitamin compound (MI group, n=20) or Folic Acid alone (FA group, n=20) for three months before COS
Participants/materials, setting, methods: Clinical characteristics and reproductive outcomes (clinical pregnancy rate and miscarriage rate) of the whole cohort of patients were recorded and analysed using t-test or chi-squared test as appropriate. Oocyte Zona Pellucida (ZP) and Meiotic Spindle (MS) morphological features were examined using Polarized Light Microscopy whereas embryo quality was assessed using the Integrated Morphology Cleavage Score evaluated on day 2 (Holte 2007) integrated with morphokinetics annotation by Time-Lapse system. Statistical significance was set at p<0.05
Main results and the role of chance: The clinical characteristics of the patients and the outcome of their IVF cycles showed no statistically significant differences. A total number of 324 oocytes were observed under PLM revealing a significantly higher zona retardance (2.1±0.6 vs 1.9±0.5; p<0.0001), area (2895.3±574.4 vs 2574.8±491.1;p<0.00001) and thickness (5.3±1.4 vs 4.5±1.2; p<0.05) and a significantly shorter meiotic spindle axis (11.8±1.8 vs 12.3±2.3) in MI group (n=155) compared to FA group (n=169).

Overall, 176 embryos were obtained and assessed for conventional morphology showing a significantly higher morphological score on day 2 (7.4±2.3 vs 6.7±2.1; p<0.01) and a higher percentage of top quality embryos (score≥8) (45.2% vs 26.1; p<0.01) in MI group (n=84) compared to FA group (n=92). At variance, comparable embryo morphokinetic parameters were observed in the study groups: time of pronuclear appearance (tPNa), pronuclear fading (tPNf), completion of cleavage to two, three, four and eight cells (t2, t3, t4, t8), time between first and second cytokinesis (P2) and between second and third cytokinesis (P3).

After ultrasound-guided ET, the implantation rate was 30% (10/30) in MI group and 11% (3/28) in FA group, showing significant differences (p<0.05) and an ongoing pregnancy rate of 40% (8/20) in MI group and 5% (1/20) in FA group (p<0.01)**Limitations, reasons for caution:** The conclusions of this study may not be generalizable to other patient populations, time-lapse systems or microscopy. The number of observations is limited and the conclusions should be verified in a prospective study on a larger number of embryos
Wider implications of the findings: This is the first, preliminary evaluation of the influence of a multivitamin compound on oocyte morphological features and embryo morphokinetics. If confirmed on a larger sample size, our preliminary findings may help to a better oocyte and embryo selection in overweight patients
Trial registration number: not applicable

P-260 Post-warming culture duration and effect on live birth rates in single, top-quality vitrified blastocyst transfers – A retrospective analysis of 839 transfers.

H. Premannandan[1], S. Yasmin[1], G. Sotirchou[1], G. Srivastava[1]

[1]Homerton University Hospital, Women's and Sexual Health, Homerton, United Kingdom

Study question: Do live birth rates differ based on the post-warming culture duration in top-quality, single vitrified blastocyst transfers in good prognosis women?
Summary answer: Live birth rates were similar in top-quality, single vitrified blastocyst transfers cultured ≤ 2 hours, between 2 to 3 hours and > 3 hours post-warming.
What is known already: It is generally accepted that the post-warming culture duration should be a minimum of 2 hours before the transfer. However, the question of difference in live birth rates, when transferring a viable blastocyst that was of top-quality at vitrification, before or after the recommended post-warming culture duration, has been not fully answered.
Study design, size, duration: Retrospective cohort study of 839 frozen blastocyst transfers in a single UK centre (March 2015 - November 2018). Post-warming culture duration of ≤ 2 hours (Group A, n=208), between 2-3 hours (Group B, n=396) and > 3 hours (Group C, n=235) were compared to live birth rates. For power of 90% with 95% CI, demonstrating medium effect size, minimum 141 participants were needed in each group for statistical significance using Chi-Square ($\chi 2$) test of independence.
Participants/materials, setting, methods: Non-smoker women with BMI less than 30, age ≤ 37 years at oocyte retrieval, having a single, top-quality blastocyst at vitrification were included. All women had hormone replacement therapy (HRT) for the preparation of the endometrium. Standard dose of micronized progesterone pessaries was used for luteal phase support. Outcome measure was live birth rates.
Main results and the role of chance: In total, 839 transfers were analysed. (i) The 3 groups of post-warming culture duration intervals yielded live birth rates of 32.69%, 38.64% and 39.15% respectively. No statistical difference between the groups was found [X^2 (2, N = 839) = 2.534, p = 0.2817].
Post-warming culture duration does not impact the chance of live birth rates in single, top-quality vitrified/warmed blastocyst transfers in good prognosis patients.**Limitations, reasons for caution:** Retrospective nature of the study. The expansion of the blastocysts has not been considered in this study; however, only viable embryos were transferred.
Wider implications of the findings: In busy IVF units, top-quality vitrified blastocysts can be transferred between 2 to 4 hours without any detrimental effect on the live birth rate.
Trial registration number: not applicable

P-261 Post warming re-expansion of single top-quality blastocysts and the relationship with live birth rates (LBR) – A retrospective analysis of 839 frozen embryo transfers

G. Srivastava[1], S. .yasmin[1], H. .premannandan[1], G. Sotirchou[1], R. Homburg[1]

[1]Homerton University Hospital - E9 6SR, Fertility Department, London, United Kingdom

Study question: Do the live birth rates (LBR) differ based on the post-warming culture duration in top-quality, single blastocyst transfers in good prognosis women?
Summary answer: LBR differ significantly based on the re-expansion of vitrified top-quality blastocyst cultured post-warming for ≤ 2, 2 to 3 and ≥ 3 hours.
What is known already: Re-expansion of the vitrified blastocyst is accepted to give an indication of its implantation potential and reproductive outcomes. Failure to re-expand affects 25% of warmed blastocysts. Nonexpanded, viable, blastocysts are deemed suitable for transfer within 4 hours of post-thaw culture in many units across the world. Warming of another blastocyst is rarely justified unless substantial damage to the trophectoderm is observed.
Study design, size, duration: Retrospective cohort study of 839 frozen embryo transfers conducted in single UK centre from March 2015 to November 2018. The post-warming culture duration was divided into three groups: ≤ 2 hours (Group A, n1 = 61 n2 = 147), between 2-3 hours (Group B, n1 = 106, n2 = 290) and ≥ 3 hours (Group C, n1 = 54, n2 = 181). LBR were compared between non-expanded (n1) and expanded (n2) group respectively.
Participants/materials, setting, methods: Non-smoking women, with BMI I< than 30, age ≤ 37 years at oocyte retrieval, having a single, top-quality embryo at vitrification were included. All women had hormone replacement therapy (HRT) for the preparation of the endometrium. A standard dose of micronized progesterone pessaries were used for luteal phase support. The outcome measure was the live birth rate.
Main results and the role of chance: In total, 839 transfers were analysed with non-expanded and expanded status with an enrollment ratio of approximately 1:2.5. The study was powered at 80% with a 95% confidence interval. To demonstrate high effect size at least 143 total participants (n1+n2)were needed for each of the time intervals respectively.

The proportion tests, two-sided Z-tests at 5% level of significance for each group confirmed that the differences in live birth rates between non-expanded and expanded status at different time intervals are statistically significant (Group A: non-expanded 21.31% vs expanded 37.41%, p = 0.024; Group B: non-expanded 30.19% vs expanded 41.72%, p = 0.03; Group C: non-expanded 22.22% vs expanded 44.20%, p = 0.003)
Limitations, reasons for caution: The study was retrospective in nature.
Wider implications of the findings: In busy units, top-quality, warmed, viable, non-expanded blastocysts can be transferred between 2 to 4 hours with acceptable LBR. However,re-expanded blastocysts increased LBR significantly. We recommend, if the blastocyst has not re-expanded within 4 hours, there can be a discussion with the patient to warm remaining blastocyst to improve LBR.
Trial registration number: Not applicable

P-262 The effect of culture media microdroplet geometry and different oil overlay on pre implantation embryonic development in dry incubators: Prospective randomized study

M. Hozaien[1], A. El shimy[1], H. El khedr[1], H. Zaki[2]

[1]Ganin Fertility Center, IVF Laboratory, cairo, Egypt ;
[2]Ganin fertility center, Clinic, Cairo, Egypt

Study question: Do the culture media microdroplet geometry of different culture dishes and oil overlay affect the pre implantation embryo development in dry incubators?
Summary answer: Pre implantation embryonic development in dry incubators could be affected significantly by the geometry of culture media microdroplet and the oil overlay used.
What is known already: Commonly used embryo culture dishes are not developed especially for embryological purposes. There is a little work of physical embryonic requirements and culture platform role on human embryonic development. Different drop geometries(2D/3D dishes)results in different surface areas, evaporation rates and osmolality of media microdroplets. Oil types with different viscosities, densities and water content used for media overlay may result in changes of media evaporation rate, pH and osmolality especially in dry incubators. Minor changes in media's pH or osmolality has a dramatic effect on embryo metabolism, developmental competence and quality. Especially the blastulation rate that's much affected by high pH/osmolality.
Study design, size, duration: A prospective randomized study included 1695 MII sibling oocytes collected from 100 patients undergoing ICSI at private center from August 2019 to January 2020. We used sibling oocytes splits between two different dishes : GPS (life global, USA) and SPL (life science, Korea) with two different oils : light mineral oil (LM)(Irvine, USA) and paraffin oil (PO)(Vitro life, Sweden). All embryos cultured in dry incubators at the same conditions.
Participants/materials, setting, methods: Maternal age is ≤37 years old with ≥ 8 MII oocytes. First, we split (211 MII) between GPS and SPL dishes overlaid with LM, Other (435 MII) splits overlaid with PO, second: (176 MII) siblings were cultured in GPS dishes and splits between LM and PO, (416 MII) cultured in SPL dishes and splits between LM and PO. And, third: (457 MII) splits were cultured in GPS overlaid with PO vs SPL overlaid with LM.
Main results and the role of chance: Data collected and analyzed using statistical software, results considered significant if p value ≤ 0.05.There were no significant differences in fertilization, cleavage rates or high-quality day 3 embryos in all groups. There was significant difference in blastulation rate (75%,60%) (p = 0.02) between GPS dishes vs SPL dishes overlaid with LM respectively. GPS dishes overlaid with PO showed significantly higher blastulation rate (67% vs 47%) (p<0.001) and high-quality day 5 embryos (75%,48%) (p <0.001) than SPL overlaid with PO. On the other hand, there were no significance in SPL splits between LM vs PO overlay in any recorded embryological parameters. In GPS splits between LM vs PO groups, the only significance was in high quality day 5 embryos (51%,32%) (p = 0.01) respectively. the combined system of GPS with PO vs SPL with LM showed significant increase in high quality day 5 embryos (75%,62%) (p = 0.003) respectively, and tend to be significant in High quality day 3 embryos (75%,67%) (p = 0.06) respectively. So, media drop geometry of 3D dishes combined with paraffin oil lead to higher pre implantation embryonic development with higher morphological quality.
Limitations, reasons for caution: Small sample size and the morphological assessment would be better if combined with morphokinetics or PGT-A results, especially aneuploidy, mosaicism and deletion vs addition in the chromosomes. The study needs to be expanded to include ongoing pregnancy rate.
Wider implications of the findings: If these findings backed with larger prospective randomized trials to reach statistically powered results, the impact of dry conditions on embryonic culture media could be overcame by using 3D dishes overlaid with heavy oil.
Trial registration number: Not applicable

P-263 Validation of a deep convolutional neural network trained to assess whether an embryo meets criteria for biopsy/cryopreservation using a test set of PGT embryos

I. Souter[1], S. Vagios[1], C. Sacha[1], M.K. Kanakasabapathy-[2], P. Thirumalaraju[2], C. Bormann[1], H. Shafiee[2]

[1]Massachusetts General Hospital-Harvard Medical School, Obstetrics/Gynecology-Reproductive Endocrinology and Infertility, Boston-MA, U.S.A. ;
[2]Brigham & Women's Hospital-Harvard Medical School, Medicine/Division of Engineering in Medicine, Boston, U.S.A.

Study question: Can a deep convolutional neural network (CNN) decide whether a Day-5 (D5) embryo, previously assisted hatched (AH) for preimplantation genetic testing (PGT), meets biopsy/cryopreservation criteria?
Summary answer: The CNN exhibited an outstanding performance in identifying, among AH embryos, D5 blastocysts that met developmental criteria for biopsy and cryopreservation.
What is known already: Embryologists make procedural and disposition decisions based on embryo morphology. There is a high degree of variability in scoring embryos, likely due to the subjective nature of morphology grading. This leads to less precise decisions for identifying embryos that meet biopsy or freezing criteria. This issue can be compounded when artificially hatching an embryo as this procedure allows blastocysts to escape from the zona at an earlier stage of development. A deep CNN has been developed using untested embryos to make biopsy and cryopreservation decisions[1]. Could this CNN be used to accurately assess embryos that have undergone AH for PGT?
Study design, size, duration: Retrospective cohort study of 1944 embryos derived from 224 PGT cycles that took place at an academic fertility center between 8/2014 and 12/2019.

Participants/materials, setting, methods: We evaluated the ability of a CNN-based artificial intelligence platform, trained using untested embryos, to identify D5 embryos for biopsy and cryopreservation and compared it with highly-trained embryologists. All embryos were laser-AH on D3 to help facilitate trophectoderm biopsy at the blastocyst stage. The developmental criteria for D5 biopsy and cryopreservation are the same in our center: ≥ grade 3 embryos and grade C or better for inner cell mass and trophectoderm (Gardner grading system).
Main results and the role of chance: Deep CNN correctly identified which D5 embryos met criteria for biopsy and cryopreservation with sensitivity: 93.7% (95%CI: 91.6-95.3%), and specificity: 96.3% (95%CI: 95.1-97.3%), (n=1944). The positive and negative predictive values of the CNN-based artificial intelligence platform were 93.4% (95%CI: 91.3-95.1%) and 96.5% (95%CI: 95.2-97.4%), respectively (n=1944).
Our system displayed an accuracy of 95.4% (95%CI: 94.3-96.5%) (n=1944).
Limitations, reasons for caution: Images utilized were obtained using a single imaging platform (Embryoscope™) at one timepoint and were annotated using criteria from a single IVF program. Prospective trials should confirm our findings prior to wider application of this system in clinical practice.
Wider implications of the findings: Our results suggest that in embryology, CNNs can be used to automate decision-making related to classification and disposition of AH embryos with a high degree of accuracy. Future studies will utilize expanded datasets, additional time-points and clinically relevant patient/cycle variables to enhance the power and accuracy of testing algorithms.
Trial registration number: NOT APPLICABLE

P-264 Using Time-lapse imaging (TLI) to assess blastocyst collapse dynamics and Trophectoderm (TE) quality as a marker of implantation potential

R. Alhamdan[1], L. Zujovic[2], J. Hernandez Medrano[3]

[1]School Of Human Development, Obs & Gyne, Nottingham, United Kingdom ; [2]Nurture Fertility, Director of embryology, Nottingham, United Kingdom ; [3]University of Nottingham, Child health and obstetric and gynaecology, Nottingham, United Kingdom

Study question: Does the frequency and type of blastocyst collapsing impact implantation and wither blastocyst TE quality and embryo developmental kinetics influence the type of blastocyst collapsing observed
Summary answer: Frequency and type of blastocyst collapsing did not influence implantation potential. However, those blastocysts exhibit higher quality TE and a shorter time to form.
What is known already: Few studies have examined the phenomenon of blastocyst collapsing in mammals and their results appears to be contradictory. A study in mice reported that collapsing does not affect implantation when it is not extensive (≤ 20%). While another study in human, suggests that collapsing embryos have lower implantation potential. This reduction in implantation potential was present irrespective of whether the extent of collapsing pattern is week (≤50% separation of the TE) or strong (≥50%separation of the TE). Therefore a more comprehensive understanding of the type and frequency of blastocyst collapsing and its connection to implantation potential is required
Study design, size, duration: A retrospective analysis including a total of 500 embryos after IVF/ICSI and fresh blastocyst transfer between 2017/2018, in Nurture Fertility, The Fertility Partnership group, Nottingham, UK.
Participants/materials, setting, methods: Blastocyst collapsing dynamics were collected from the TLI (Embryoscope, Vitrolife). Exact traceability of transferred embryos was included in the analysis. The association between blastocyst collapsing dynamics and frequency (no collapse, ≥50% or <50% separation of the surface of the TE from the ZP), zona pellucida thickness (µm), blastocyst expansion (µm^2), and KID score were assessed. Embryo developmental kinetics and TE quality were also compared. Potential confounding factors, e.g: maternal age and stimulation protocol were considered
Main results and the role of chance: There was no significant difference in implantation potential based on the frequency or type of blastocyst collapse dynamics (no collapse, ≥50% or <50% separation of the surface of the TE from the ZP). However, collapsing blastocysts significantly correlated with thinner ZP (p =0.005). Blastocysts exhibiting ≥50% separation of the blastocoel cavity strongly correlated with the highest TE quality (graded as A in accordance with the Gardner's classification system). Timing to morula (-2.4 hr), blastulation (-1.85hr) and blastocysts expansion (-1.94hr) were significantly (p< 0.001) shorter in collapsing embryos. Irrespective to blastocysts collapse dynamic and frequency, embryos with TE graded A displayed an increased chance of a positive KID score (p< 0.004, OR and 95% CI (A vs. B 0.1651 (0.0518, 0.5266), A vs C 0.2122 (0.0652, 0.6908), A vs D 0.0611 (0.0045, .0.8311)). We observed no significant effect from potential confounding factors such as patient age or stimulation protocol
Limitations, reasons for caution: The retrospective design of the study limits its value. Another limitation is presented by the fact that one embryologist measured the blastocysts collapsing frequency and dynamics and therefore, no inter-operator quality assurance.
Wider implications of the findings: The study of blastocyst morphological dynamics may help to improve the selection criteria of blastocyst with higher potential to implant especially for those patients with poor prognosis.
Trial registration number: not applicable

P-265 For blastocyst frozen embryo transfer: prolonged post-thaw culture may improve transfer outcomes

S.K. Nguyen[1], K.C. Do[1], T.T.C. Bach[1], A.T. Vu[1], V.T. Phung[1], T.L. Dinh[1]

[1]Buu Dien Hospital, ART center, Hanoi, Vietnam

Study question: In comparison with two hour post-thaw culture, are the blastocyst transfer outcomes of prolonged culture (16 ± 1 hour) better or not?
Summary answer: Prolonged post-thaw culture resulted in better blastocyst transfer outcomes: significantly higher implantation rate and significantly increased clinical pregnancy rate.
What is known already: Frozen blastocysts have been commonly transferred two to four hours after thawing. However, another school of thought has advised that prolonged post-thaw culture of blastocysts for more than 20 hours could assist in monitoring the post-thaw development of blastocysts, leading to better embryo selection for transfer; thus, better outcomes ultimately. In addition, embryos that underwent two freeze–thaw cycles may experience more stress than embryos that underwent one cycle. Here, we examine the correlation between post-thaw culture duration of blastocysts and the transfer outcomes. We also analyse attentively this correlation of embryos that have undergone two frozen-thaw cycles.
Study design, size, duration: A retrospective study: data transfer cycles from 01/2019-01/2020 were analyzed. Transfers of one or two blastocysts were included (good and fair grade only; poor quality blastocyst transfers were excluded). Patients were divided into two groups: group L (n=112) comprises patients who had their frozen blastocysts thawed in the previous afternoon then transferred the next morning (16 ± 1 hour culture). Group S (n=100) includes cases of frozen blastocyst transfer two hour after thawing.
Participants/materials, setting, methods: The majority of cases in each group had their blastocysts vitrified on day 5 of development (one freeze-thaw cycle). However, each group has a subgroup of embryos that had been vitrified on day 2, thawed, let grow until day 5, underwent one more freeze-thaw cycle (two freeze-thaw cycles) (named L-twice: n=31 and S-twice: n=26) . No PGT involved; donations excluded. BetaHCG positive, implantation, clinical pregnancy were registered. t-test and Chi-square test were applied.
Main results and the role of chance: The average maternal ages of group L and group S were comparable (29.32 ± 4.26 and 29.98 ± 4.16 years, respectively, p>0.05). The average numbers of transferred embryos per cycle of group L and S were equal (1.32 ± 0.47 and 1.33 ±0.47, p>0.05). Group L (prolonged culture) showed significantly better outcomes than group S (short culture): positive BetaHCG rate was 73.21% vs 58.00% (p<0.05), CPR was 67.86% vs. 53.00%, and IR was 60.81% vs. 46.62% (p<0.05), respectively. Multiple pregnancy rate was comparable between two groups (13.92% for L group and 14.81% for S group), possibly due to similar average numbers of transferred embryos between two groups. Notably, for blastocysts which had undergone two freeze-thaw cycles, the correlation remains the same: prolong culture had better outcome. For L-twice, positive Beta-hCG rate, CPR and IR was 64.52%, 61.29% and 54.35%; while for S-twice, these rates were 57.69%, 57.69% and 52.63%, respectively. These data suggest that prolong culture may improve transfer outcomes of frozen blastocyst transfer cycles.

Limitations, reasons for caution: This is a retrospective study. Further prospective, randomized clinical trial is required. The sizes of subgroup S-twice and L-twice are small, extend analysis is required to collect larger sample size.
Wider implications of the findings: Previous reports on the impact of post-thaw culturing duration on transfer outcomes have been controversial. Our findings suggest that longer culture may assist blastocysts to "recover" from freeze-thaw cycles. Further analysis of the impact of prolonged culture on the epigenetics of the embryos may be valuable.
Trial registration number: not applicable

P-266 Selecting spermatozoa with the highest chromatin integrity

J. Stewart[1], A. Parrella[1], D. Tavares[1], M.S. Wang[1], M. Haddad[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: What is the impact of selecting spermatozoa with the highest chromatin integrity on ICSI outcomes?
Summary answer: We selected spermatozoa with the highest progressive motility and chromatin integrity by microfluidic sperm selection (MFSS) and achieved superior implantation and delivery rates.
What is known already: Sperm preparation methods aim at providing specimens for insemination with the highest progressive motility independent of phenotypic and genomic integrity. It has recently been recognized that a microfluidics device yielded spermatozoa with the highest progressive motility as well as superior chromatin integrity. Here we compared two sperm selection methods: density gradient centrifugation (DGC) and MFSS.
Study design, size, duration: From October 2016 to January 2020, ejaculates that were processed by DGC and MFSS for ICSI treatment from 8 consenting men were screening for DNA fragmentation by TUNEL. In addition, ejaculates from 22 men were processed solely by MFSS for ICSI treatment. Semen parameters, chromatin integrity, embryo implantation, and pregnancy characteristics were compared.
Participants/materials, setting, methods: Fresh ejaculated specimens from consenting men were collected for standard semen analysis in accordance with WHO 2010 criteria. DGC and MFSS were used to isolate motile spermatozoa based on cell motility and fluid dynamics. Sperm chromatin fragmentation (SCF) was assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) on at least 500 spermatozoa under a fluorescent microscope utilizing a threshold of ≥15%. ICSI was performed in the standard fashion.
Main results and the role of chance: A total of 20 men (43±6 years) had the following average semen parameters: concentration of 18±15 x 106/mL, 30±18% motility, and 2.4±1% morphology. After DGC and MFSS, the sperm concentration was 2.2±1 and 1.5±12.6 x106/mL, with 49±32% and 97.4±5% motility, respectively (P<0.0001).

The morphology of the raw sperm sample improved from 2.3±1% to 4.0±1% after MFSS, while it remained at 2.6±1% after DGC.

In 8 men (43±6 years), the SCF in their raw sample was 23%, falling to 18% after DCG selection and to 1.2% after MFSS (P < 0.0001). They underwent 20 ICSI cycles with their female partners, (37±3 years) with DGS sperm selection, achieving a 58% fertilization rate (80/138). The implantation rate was 5% (2/41) with an 11% clinical pregnancy rate (CPR) (2/18) and one pregnancy loss. Subsequently, ICSI with MFSS achieved a fertilization rate of 65%, a 29% (5/17; P<0.01) implantation rate, and a 63% (5/8; P<0.001) CPR. An additional 22 men (43±7 years) underwent 28 ICSI cycles solely with MFSS due to poor reproductive history. A 76% fertilization rate (203/266) and 54% (31/57) good quality embryos were achieved. The implantation rate was 26% (15/57) with a 50% CPR (13/26).
Limitations, reasons for caution: This is a preliminary study on a small number of subjects. Although we controlled for a concurrent female factor, this cannot be excluded with certainty. As this is a new method, the health of the resulting offspring would need to be evaluated to confirm the safety of the technique.
Wider implications of the findings: According to our study, SCF appears to be linked to the kinetic characteristics of the sperm cell. This novel microfluidic device may help to identify spermatozoa with the highest functional and genomic integrity and ultimately be routinely used in ART treatments.
Trial registration number: not applicable

P-267 Duration of exposure to EmbryoGlue (EG) may significantly impact implantation and clinical pregnancy rates following Embryo Transfer (ET).

R. Ebrahimi Ghaei[1], C. Harrity[2], C. Riordan[2], C. Moran[2], D. Keane[2]

[1]University of Royal College of Surgeons in Ireland RCSI, Obstetrics & Gynaecology, Dublin, Ireland ;
[2]ReproMed Ireland, Reproductive Medicine, Dublin, Ireland

Study question: Does EG increase implantation and clinical pregnancy rates in fresh transfer Assisted Reproductive Technology (ART) cycles and is the duration of exposure significant?
Summary answer: Embryos exposed to EG >30mins have significantly lower Implantation Rates (IR) and Clinical Pregnancy Rates (CPR) than those exposed <30 mins or not at all.
What is known already: Various modifications have been made to culture media in the hope of increasing success rates, including the addition of Hyaluronan (HA). EG (Vitrolife, Goteburg) is one such medium. The 2014 Cochrane review (Bontekoe et al) concluded moderate quality evidence of benefit when using HA enriched media prior to ET.

Vitrolife, recommends equilibration of embryos in EG between 10 mins – 4 hrs prior to transfer. Studies including duration of exposure as a parameter have, to date, taken 10 mins as the cut off time. We could find no study which compared the effect of different durations of exposure to EG.
Study design, size, duration: A retrospective cohort study was designed to determine if duration of exposure to EG influenced outcomes. Cycles from 01 January 2015 – 31 December 2019 were analysed.

1859 cycles reached oocyte retrieval and were screened against the inclusion/exclusion criteria to identify relevant patients.

3 study groups were chosen

Control: Transfers without EG (138)
Group 1: Transfers with EG exposure <30mins prior to ET (170)
Group 2: Transfers with EG exposure for >30mins prior to ET (295)

Participants/materials, setting, methods: The study was performed in a single university-affiliated reproductive medicine centre. Inclusion criteria were IVF/ICSI cycles resulting in fresh blastocyst transfer.
Cycles with elective freeze, cleavage transfer, failed fertilisation, or no oocytes/embryos were excluded. From 1859 retrievals, 540 patients had 603 transfers of 809 blastocysts meeting the inclusion criteria during the study period.
Primary outcome was IR, secondary outcome was Pregnancy Rate (PR). Statistical analysis was performed using Chi-square for proportions and t-test for means.
Main results and the role of chance: Control group, no EG (n=138):

- Age: 37.0
- Anti-Müllerian hormone (AMH): 15.0 pmol/l
- Number of embryos 160
- Mean number blastocyst per ET: 1.16
- PR: 0.536
- IR: 0.269
- CPR: 0.312

Group 1, EG exposure <30mins (n=170):

- Age: 37.3
- AMH: 13.8 pmol/l
- Number of embryos 199
- Mean number blastocyst per ET: 1.16
- PR: 0.547
- IR: 0.367
- CPR: 0.429

Group 2, EG exposure >30mins (n=295):

- Age: 37.4
- AMH: 14.8 pmol/l

- Number of embryos 450
- Mean number blastocyst per ET: 1.51
- PR: 0.495
- IR: 0.156
- CPR: 0.237

There was no difference between age (p=0.2) or AMH (p=0.79) between groups. Significantly more embryos were transferred in group 2 (p<0.0001).

The difference in PR between all 3 groups was not significant (p=0.5), although this may be influenced by the number of embryos transferred. Both IR (p<0.0001) and CPR (p<0.0001) were significantly lower in the group with extended exposure to EG.

Unfortunately, this was a retrospective cohort study rather than a prospective randomised controlled trial. Therefore, the impact of bias cannot be excluded.

Limitations, reasons for caution: This is a retrospective study over 5 years, during which subtle changes may have occurred in laboratory practice and personnel. The potential impact of additional confounding factors cannot be accounted for without randomisation.

Embryo quality was not included as a parameter, but exclusion of cleavage-stage transfers should limit this impact.

Wider implications of the findings: Studies to date have shown either a potential benefit or no benefit with the use of EG. Our study supports this conclusion but also suggests that exposure to EG for >30mins may be detrimental.

Trial registration number: Not Applicable

P-268 Embryo glue transfer medium for women >= 35 years of age - outcome in fresh IVF/ICSI cycles

V. Tomic[1], M. Sommergruber[1]

[1]University hospital SALK Paracelsus Medical University, Gynaecology and Obstetrics, Salzburg, Austria

Study question: Add-on embryo glue in single embryo transfer in the women ≥ 35 years of age, does it make a difference?

Summary answer: Results showed beneficial effect of embryo glue medium in women ≥ 35 years of age and those with recurrent implantation failure.

What is known already: A Cochrane review of 6 RCTs by Bontekoe and al. found higher live birth rate using embryo glue (OR 1.41) (Bontekoe et al., 2014). However, the incidence of multiple pregnancies was also increased significantly (OR 1.86).

Afterwards, study by Signh et al.from 2017 and Fancovits et.al from 2015 did not found statistically significant difference in the clinical pregnancy rate with the use of embryo glue medium. However, the most of te studies are statistically powerless because of a small sample size and/or different criteria, and studies with a SET are currently lacking.

Study design, size, duration: A retrospective cohort study on totally 680 patients, divided into two groups undergoing IVF/ICSI, in the tertiary University center between 2013-2019.

Participants/materials, setting, methods: In total 295 patients in the study group, embryos were transferred into 50µL of Embryo-glue for 15 minutes prior to transfer and control group where embryo were transferred to conventional blastocyste culture medium. Statistical analysis was performed by SPSS ver. 22 for Windows.

Main results and the role of chance: Clinical pregnancy rate in the study group with the use of hyaluronan enriched medium (Embryo-glue) was significantly higher than the control group (36.5% vs 28.3%, p<0.05). When we analyzed subgroup of women with recurrent implantation failure, statistical difference was even more powerful with p<0.01. However, our results did not found difference in the number of retrieved eggs, days of stimulation, total dose of gonadotrophins, type of fertilization (IVF or ICSI), fertilization rate or miscarriage rate. Multiple pregnancy rate was also similar between two groups.

Limitations, reasons for caution: Limitations of the current study include retrospective design and as such bias can not be excluded. Also our study excluded women in favorable maternal age (younger then 35 years of age) so effect on embryo glue in this age group could not be commented.

Wider implications of the findings: Results of current study showed beneficial effect of Embryo-glue in selected group of patients, women in advanced maternal age and recurrent implantation failure but future RCT studies are necessary to evaluate impact of Embryo-glue on all patients.

Trial registration number: Not applicable

POSTER VIEWING SESSION

ENDOMETRIOSIS, ENDOMETRIUM AND AND FALOPIAN TUBE, AND BENIGN DISORDERS OF THE ENDOMETRIUM AND FALLOPIAN TUBE

P-269 Cumulative live birth rates after IVF-ICSI cycles in women with endometriosis-associated infertility: prolonged GnRH-agonist versus GnRH-antagonist protocols

H. Wei[1], Y. Chen[1], X. Li[1], Y. Lin[1], R. Li[1], C. Ma[1]

[1]Peking University Third Hospital, Center for Human Reproductive Medicine, Peking, China

Study question: Are cumulative live birth rates similar in prolonged GnRH-agonist and GnRH-antagonist protocols after IVF-ICSI cycles in women with endometriosis-associated infertility including all subsequent frozen–thaw cycles from the same oocyte retrieval?

Summary answer: No differences were found in cumulative live birth rates(-CLBRs) after one complete IVF-ET cycles between prolonged GnRH-agonist and GnRH-antagonist protocols in women with endometriosis-associated infertility.

What is known already: Endometriosis is known to have an impact on fertility. Women with endometriosis often require in vitro fertilization (IVF) to improve the chance of pregnancy. A reduced ovarian reserve was demonstrated in patients with endometriosis. Multiple studies of the prolonged GnRH-agonist and GnRH-antagonist protocols on pregnancy rate and live birth rate have yielded controversial findings. A 2006 systematic review of 27 RCTs showed that GnRH-antagonist protocol has a significantly lower clinical pregnancy rate and live birth rates than those in GnRH-agonist long protocol. However, a 2016 systematic review of 73 RCTs concluded that these two protocols have equivalent live birth rates.

Study design, size, duration: A retrospective case-control study comprised women with endometriosis who underwent IVF-ICSI cycles between January 2015 to December 2016, at the Reproductive Medical Center of Peking University Third Hospital. Propensity score matching (matching ratio 1:1, caliper width = 0.02) was used to create a comparable control group, including 303 women with prolonged GnRH-agonist protocol and 303 women with GnRH-antagonist protocol.

Participants/materials, setting, methods: A retrospective case-control study comprised women with endometriosis who underwent IVF-ICSI cycles between January 2015 to December 2016. Propensity score matching (matching ratio 1:1, caliper width = 0.02) was used to create a comparable control group, including 303 women with prolonged GnRH-agonist protocol and 303 women with GnRH-antagonist protocol.Cumulative live birth rates (CLBRs) upon the first complete IVF-ICSI cycles, including transfers of all resulting embryos was considered as a primary outcome measure.

Main results and the role of chance: Baseline characteristics including BMI, age, basal FSH, duration of infertility were comparable in the prolonged GnRH-agonist group and the GnRH-antagonist group. Patients in the prolonged GnRH-agonist group had a significantly higher gonadotropin consumption (3487.55 (1200-10425) vs. 2175.00 (600-5550)(IU), P<0.01) and a longer time of stimulation (13.00(8.00-24.00) vs. 10.00(4.00-19.00)(days), P<0.01) than that in the GnRH-antagonist group. There's no significantly difference between two groups of the estradiol levels on hCG trigger day (7087.00(525.00-29976.00) vs. 5995.00(676.00-26075) (pmol/mL), P=0.06). The number of oocytes retrieved per cycle and number of usable embryos achieved were comparable between two groups. The implantation rate in the GnRH-antagonist group after fresh ET was significantly lower (28.5% vs. 34.9%, p =0.047) compared with prolonged GnRH-agonist group. A GnRH-antagonist protocol appears to result in lower rates of clinical pregnancy and live birth after fresh ET (40.8% vs. 46.5%, p = 0.22; 33.2% vs. 39.1%, p = 0.19, respectively), but the difference of which did not reach

statistical significance.The cumulative clinical pregnancy rates and CLBRs were comparable for the prolonged GnRH-agonist group and the GnRH-antagonist group with 55.1% vs. 52.8%, (odds ratio(OR): 0.91; 95% CI: 0.66-1.25; P=0.57) and 45.5% vs. 43.6%, (OR: 0.92; 95% CI: 0.67-1.27; P=0.62), respectively.

Limitations, reasons for caution: One limitation of this study is its retrospective nature, however propensity score matching was selected to create control cohorts for a better matched analysis. The reproductive outcomes in different pathological types of endometriosis were not analyzed, such as peritoneal endometriosis, deep infiltrating endometriosis or endometrioma.

Wider implications of the findings: A GnRH-antagonist protocol appears to result in lower rates of implantation, clinical pregnancy and live birth after fresh ET, suggesting that a GnRH- antagonist protocol might negatively impact endometrial receptivity. However, we find similar CLBRs between the two groups in women with endometriosis-associated infertility.

Trial registration number: not applicable

P-270 Fibronectin (FN1) and Progranulin (GRN) expression in eutopic endometrium – potential biomarkers among different stages of endometriosis (EM)

I. Holzer[1], A. Machado Weber[1], A. Marshall[1], A. Freis[1], T. Strowitzki[1], A. Germeyer[1]

[1]University Hospital Heidelberg, Dept. of Gynaecological Endocrinology and Fertility Disorders, Heidelberg, Germany

Study question: Does the local endometrial gene and protein expression of FN1 and GRN vary among different degrees of EM?

Summary answer: Significant differences in gene and protein expression were identified not only between women with and without EM but also among different levels of the disease.

What is known already: Endometriosis is a common gynecological disease affecting 10 – 15 % of women of reproductive age. However, molecular mechanisms and pathogenesis are still not completely understood. Due to the absence of a reliable clinical biomarker, the only viable method for the often delayed definitive diagnosis is laparoscopic surgery. FN1 is a glycoprotein, which is involved in cell adhesion, migration and implantation. GRN, a protein with proliferative and invasive properties, seems to be involved in embryogenesis. Our objective was to investigate molecular mechanisms of these two genes and proteins among various stages of EM and analyze their potential as diagnostic markers.

Study design, size, duration: This study analyzed eutopic endometrial tissue of women suffering from different stages of EM (n = 58). Our aim was to investigate changes in endometrium of women with EM compared to healthy endometrium as well as among different levels of EM (minimal, mild, moderate) to detect potential new biomarkers for the disease.

Participants/materials, setting, methods: Endometrial biopsies were taken from women undergoing a laparoscopic surgery for benign reason. The expression on mRNA level of *FN1* and *GRN* was analyzed using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Protein expression of the proteins was determined using Enzyme-linked immunosorbent assay (ELISA) as well as the Avidin-Biotin method to stain cryosections for immunohistochemistry.

Main results and the role of chance: Significant differences in gene and protein expression of both examined genes were identified not only in eutopic endometrial tissue of women with EM compared to women without EM but also among different levels of the disease ($p < 0.05$). The strongest mRNA expression of both *FN1* and *GRN* was seen in women with minimal EM. However, protein expression of FN1 and GRN was significantly reduced in women with EM compared to women without EM. More precisely, the magnitude of the protein expression decreased with the severity of the disease ($p < 0.05$). While the protein expression of FN1 was mainly noticed in stromal and endothelial cells, GRN was mostly present in stromal and epithelial cells.

Limitations, reasons for caution: The main limitation of this study is its small sample size. A larger study population would be needed to validate the results.

Wider implications of the findings: These findings of different gene and protein expression between different levels of EM may support the idea of a distinct pathophysiology between mild and severe EM and suggest the potential use of FN1 and GRN as new clinical biomarkers.

Trial registration number: not applicable

P-271 Effects of chronic endometritis on fertility and pregnancy outcomes

A. Morimune[1], F. Kimura[1], A. Nakamura[1], J. Kitazawa[1], T. Hanada[1], K. Hirata[2], A. Takebayashi[3], A. Takashima[1], S. Kaku[1], S. Moritani[4], R. Kushima[4], T. Murakami[1]

[1]Shiga University of Medical Science, Department of Obstetrics and Gynecology, Shiga, Japan ;
[2]Goto Ladies Clinic, Division of Reproductive Medicine, Osaka, Japan ;
[3]National Hospital Organization Higashi-ohmi General Medical Center, Department of Obstetrics and Gynecology, Shiga, Japan ;
[4]Shiga University of Medical Science, Department of Clinical Laboratory Medicine and Division of Diagnostic Pathology, Shiga, Japan

Study question: Does chronic endometritis (CE) affect fertility and the course of pregnancy in patients without implantation failure, habitual abortion, and diseases suspected to cause implantation failure?

Summary answer: Patients with CE have lower pregnancy and live birth rates and higher miscarriage and preterm birth rates.

What is known already: Several reports have demonstrated that CE is associated with infertility and implantation failure. However, these clinical researches have mainly involved patients with recurrent implantation failure (RIF). The subsequent pregnancy rate after the diagnosis of RIF is considered to be low even without CE. Thus, RIF without CE cannot be used as the control group to examine the effects of CE on fertility and pregnancy outcomes. In the present study, patients with RIF, recurrent pregnancy loss (RPL), and diseases suspected to cause implantation failure were excluded, and the effects of CE on fertility and pregnancy outcomes were determined.

Study design, size, duration: This retrospective study reviewed a registration list of patients attending our hospital for infertility and histologically diagnosed with the presence or absence of CE from September 2013 to December 2017 to identify study patients. Patients were then followed up based on their medical charts for 1 year after the diagnosis of CE.

Participants/materials, setting, methods: Patients were treated with IVF for a year after the histological diagnosis of CE. Endometrium was collected 5–9 days after ovulation. The pregnancy and pregnancy outcome rates were analyzed. Those who received antibiotics for >7 days to treat CE, aged >40 years, or had RIF, RPL, and a suspected cause of implantation failure were excluded from the study.

Main results and the role of chance: A total of 40 non-CE and 44 CE patients were finally analyzed.

Percentages of pregnancies and live births were as follows: 95% vs. 72.7%, $P < 0.01$; 85% and 45.4%, $P < 0.0003$ in the non-CE and CE groups, respectively. Percentages of miscarriages in pregnancy were 12.8% vs. 40.0%, $P < 0.03$, whereas percentages of preterm births in ongoing pregnancy were 2.9% vs. 23.8%, $P < 0.03$ in the non-CE and CE groups, respectively.

In the logistic analysis, CE was a factor adversely affecting objective pregnancy variables associated with live births in all patients, miscarriage in pregnancy, and preterm birth in ongoing pregnancy.

Limitations, reasons for caution: This was a retrospective study; hence, inevitable limitations are observed. Patients who received antibiotic treatment for CE were excluded. Patients who are seeking antibiotic treatment might have a long history of infertility treatment and/or might have more refractory infertility. These patients were excluded only from CE.

Wider implications of the findings: Pregnancy and live birth rates within a year after the diagnosis of CE were lower in patients undergoing IVF not diagnosed with RIF, RPL, or a disease suspected to cause implantation failure. These findings suggest that patients undergoing IVF should be examined for CE.

Trial registration number: R2014-090

P-272 The pattern of vaginal/endometrial microbiome as a predictor for outcome of in vitro fertilization (IVF) in patients with or without repetitive implantation failure:a pilot study

A. Bernabeu[1], B. Lledo[2], M.C. Diaz[3], F. Lozano[2], A. Rodriguez-Arnedo[3], R. Morales[2], R. Bernabeu[1]

[1]Instituto Bernabeu, Reproductive Medicine, Alicante, Spain ;
[2]IB Biotech, Molecular Laboratory, Alicante, Spain ;
[3]Instituto Bernabeu, IVF Lab, Alicante, Spain

Study question: Is the pattern of certain vaginal/endometrial bacteria associated with failure or success to achieve pregnancy after IVF in patients with or without repetitive implantation failure?
Summary answer: The vaginal and endometrial microbiome pattern correlate with the pregnancy rate, being different between patients who suffer or not repetitive implantation failures.
What is known already: Thanks to an improved technology in the last decade as for instance, mass sequencing, a progress in the study of microbial communities was made. Successful embryo transfer depends on many factors, including the presence of microbial colonization of the upper genital tract. The vaginal microbiome plays an important role in the maintenance of the health of the woman. Currently more and more patients are using assisted reproduction techniques, which should be offered a multidisciplinary approach for diagnosis. The aim of this study is to investigate if the vaginal and endometrial microbiome in couples undergo ART could affect the pregnancy rate.
Study design, size, duration: Prospective analysis including 48 patients (18-50 years) recruited from May 2017-May 2019 (264 samples). Of these, 23 patients experienced unexplained repetitive implantation failure (RIF). All patients performed PGT-A and single embryo cryotransfer. Vaginal samples were collected on the immediate pre-transfer cycle (artificial endometrial preparation) (visit 1), on the day of the transfer (visit 2) and on the day of the pregnancy test (visit 3). Endometrial samples were taken on the immediate pre-transfer cycle (visit 1).
Participants/materials, setting, methods: DNA extraction was performed using the PureLink Microbiome DNA Purification kit. Sequencing the rRNA16S V3V4 region analysis, it was carried out according to Illumina Metagenomics protocol using the NexteraXT library on the Miseq instrument. The bioinformatic tools QIIME2, MicrobiomeAnalyst and Phyloseq have been used to determine the composition of the microbiome.
Main results and the role of chance: Regarding to the microbiome dynamics during the cycle, we observed a decrease in α-diversity from the follicular to luteal phase in the study group, in contrast to a stability of the pattern in the RIF group. As for endometrium and vagina microbiome, there were α-diversity differences (Shannon p=0.0139, Simpson p=0.046), being higher for endometrium; and also β-diversity was different (p=0.001). A greater relative abundance was observed in vagina of Lactobacillus spp., (83.17% vs 84.82%,p<0.0001), Streptococcus spp. (1.59% vs 7.74%,p=0.014) and Ureaplasma spp. (0% vs. 0.89%,p=0.006). Besides, it was lower for Delftia spp. (0.95% vs 0%,p=0.0003), Anaerobacillus spp. (1.59% vs 0%,p=0.0004), and Ralstonia spp. (3.17% vs 0%,p=0.0006). Concerning RIF, we observed differences in α-diversity for the study and RIF group (Shannon p =0.0206, Simpson p=0.0206). Moreover, we were able to observe significant differences in β-diversity between both groups. Also, we found differences for Ralstonia spp., (0.09% RIF and 0.73% control, p=0.0012). Finally, as for pregnancy rate, we found a difference between patients that did not achieve[JCCF1] or achieved a pregnancy for Lactobacillus spp. (91% vs 99%,p=0.0445 visit 1, 94.63% vs. 97.69%,p=0.0268 visit 2, 97.73% vs 99.74%,p=0.0492 visit 3) and L.reuteri (0.39% vs 0.17%,p=0.0397 visit 1, 0.15% vs 0.30%, p=0.0491 visit 3).
Limitations, reasons for caution: Future larger sample size studies are warranted to corroborate our initial results.
Wider implications of the findings: The lack of dynamism in the microbioma pattern of RIF patients might reflect a impaired adaptation to endometrial changes. A greater relative abundance of Lactobacillus spp. and L.reuteri is correlated with higher chances of pregnancy.
Trial registration number: NCT03153787

P-273 Is the fertility enhancing effect of hysterosalpingography with oil-based contrast temporary or long-lasting?

N. Van Welie[1], K. Rosielle[1], K. Dreyer[1], J. Van Rijswijk[1], C.B. Lambalk[1], N. Van Geloven[2], V. Mijatovic[1], B.W. Mol[3], R. Van Eekelen[4]

[1]Amsterdam UMC- Vrije Universiteit Amsterdam, Reproductive Medicine, Amsterdam, The Netherlands ;
[2]Leiden University Medical Center, Biomedical Data Sciences, Leiden, The Netherlands ;
[3]Monash University, Obstetrics and Gynaecology, Melbourne, Australia ;
[4]Amsterdam UMC- University of Amsterdam, Reproductive Medicine, Amsterdam, The Netherlands

Study question: Does the fertility enhancing effect of tubal flushing during hysterosalpingography (HSG) with oil-based contrast change over time?
Summary answer: The hazard ratio for ongoing pregnancy of oil-versus water-based contrast was 1.71 immediately after HSG, then decreased and plateaued towards 1 after approximately two years.
What is known already: HSG with oil-based contrast results in a 10% higher absolute ongoing pregnancy rate after six months when compared to an HSG with water-based contrast. In the long-term follow up study, this effect remained present 3-5 years after the HSG, albeit that the difference was reduced to 5% after additional IVF in both groups. The fertility enhancing mechanism of oil-based contrast remains unclear. Both immunological effects on the endometrium and peritoneum or a possible mechanical effect by dislodging debris or mucus plugs from the fallopian tubes have been proposed, which suggests a temporary or a lasting effect, respectively.
Study design, size, duration: We performed a secondary analysis of the H2Oil (long term follow-up) study, a multicenter randomized controlled trial among 1,119 women evaluating the effectiveness of oil-based contrast versus water-based contrast during HSG. Here, we investigate whether the fertility enhancing effect of oil- versus water-based contrast on ongoing pregnancy changed over time or remained stable.
Participants/materials, setting, methods: Women undergoing fertility work-up were included in the H2Oil study. We used a Cox proportional hazards model for time to pregnancy for a maximum of three years of follow-up, then evaluated whether the assumption of 'no change of effect over time' holds using plots and tests on the scaled Schoenfeld residuals and by assessing interactions between treatment and time. We repeated the analysis in a subgroup of women who experienced pain during HSG (Visual-Analogue-Scale-score ≥6).
Main results and the role of chance: Data on 1107 couples were available. Of these, 550 couples had oil-based contrast and 557 had water-based contrast at HSG. Ongoing pregnancy rates after 3 years were 77% and 71%, respectively. Median follow up was 9-10 months (5th-95th percentile: <1-36). The hazard ratio for ongoing pregnancy of oil versus water over three years of follow up was 1.26 (95%CI: 1.10-1.44). On the scaled Schoenfeld residual plots, we found a decrease of the hazard ratio that was linear with log-transformed time. After including an interaction with log-transformed time, we found that the hazard ratio immediately after HSG was 1.71 (95%CI: 1.27-2.31) and reduced to 1.06 (95%CI: 0.86-1.30) at approximately two years of follow up. There was no evidence for a change in hazard ratio over time in a subgroup of women who experienced pain during HSG.
Limitations, reasons for caution: This analysis was performed using data from women with unexplained or mild male infertility. The results should not be generalized to women with ovulatory dysfunction, women older than 39 years or women at high risk for tubal pathology based on their medical history.
Wider implications of the findings: The effect of oil-versus water-based contrast decreased in the first year after HSG to no effect after approximately two years. In women who experienced pain during HSG, the effect might last longer. Both results seem to favour the hypothesis that oil-based contrast may dislodge debris or mucus from fallopian tubes.
Trial registration number: Netherlands Trial Register NTR 3270 (H2Oil study), NTR 6577 (H2Oil follow-up study), www.trialregister.nl

P-274 Functional analysis of the histidine N-methyltransferase SETD3 in endometriosis

M. Götte[1], M. Poloczek[1]

[1]Muenster University Hospital, Gynecology and Obstetrics, Münster/Westf., Germany

Study question: Does the novel actin methyltransferase SETD3 have an impact on invasive growth of endometriotic cells?
Summary answer: SETD3 affects invasive growth, cell motility and contractility of endometriotic cells in vitro.

What is known already: Endometriosis is a common, benign and chronic disease in women of reproductive age that is characterized by invasive growth of endometrial tissue outside the uterus. The novel actin-specific histidine N-methyltransferaseprevents primary dystocia and modulates actin function as a prerequisite for cell motlilty, contractility and invasive growth.
Study design, size, duration: This is an in vitro laboratory study on the immortalized endometriotic cell line 12Z and primary endometriotic stroma cells. Cells were subjected to siRNA-mediated knockdown of SETD3 or a negative control siRNA, and the impact on cell motility, contractility, invasiveness, cytoskeletal structure and gene expression were studied.
Participants/materials, setting, methods: Primary endometrial stroma cells and 12Z human immortalized endometriotic cells were used for in vitro experiments. SETD3 was knocked down by siRNA transfection with two different SETD3 siRNAs. Gene expression analysis was performed by real-time qPCR. Changes at the protein level were analysed by Western blotting, ELISA and immunofluorescence. Cell migration was investigated by scratch wound healing assay. To assess cytoskeletal functions, a collagen contraction assay was performed. Invasive growth was assessed by Matrigel assay.
Main results and the role of chance: Knockdown of SETD3 was confirmed by qPCR and Western blotting. Only moderate changes in cytoskeletal element gene expression were observed after SETD3 knockdown, whereas VEGF expression was downregulated by >40% in both experimental systems ($p<0.05$, $n>3$). At the functional level, SETD3 depletion resulted in a delay in cell motility in scratch wound assays ($p<0.05$, $n>5$), in a reduction in invasive growth of 12Z cells by 50% ($p<0.05$, $n>5$) and in a reduced capability to contract collagen gels ($p<0.05$, $n>5$). Immunofluorescence microscopy for actin revealed an altered cytoskeletal structure and cell morphology.
Limitations, reasons for caution: This is a transfection-based in vitro strudy, which needs to be confirmed in more complex experimental models.
Wider implications of the findings: The presented data suggest that methylation of beta-actin, as mediated by SETD3, contributes to the remodelling of the cytoskeleton that plays a key role in cell migration and invasion. A dysregulation of SETD3 could play a role in the process of invasive growth in endometriosis.
Trial registration number: MedK

P-275 Generalized estimating equation analysis for assessing the association between the quality of life and pregnancy outcomes of IVF treatment in patients with endometriosis-related infertility

M.H. Wu[1], H.T. Ou[2], C.W. Lin[1]

[1]College of Medicine and Hospital- National Cheng Kung University, Obstetrics and Gynecology, Tainan City, Taiwan R.O.C. ;
[2]College of Medicine and Hospital- National Cheng Kung University, Pharmacy, Tainan City, Taiwan R.O.C.

Study question: Are the quality of life (QoL) of endometriosis-related infertility women measured during IVF treatment related to the pregnancy outcomes following the treatment?
Summary answer: Improving the QoL during IVF treatment in women with endometriosis-related infertility may increase subsequent pregnancy rates.
What is known already: Generalized estimating equation (GEE) analysis that accounts for the dependence of observations from same person can minimize the potential biases commonly seen in longitudinal cohort studies, especially the studies of the IVF data that comprised a series of repeatedly measured pregnancy outcomes and associated patient characteristics from multiple embryo transfer cycles in same study subject. From emotional aspect, psychological counseling for patients undergoing IVF treatment might improve their QoL and enhance subsequent IVF pregnancy rates. In addition, oxytocin antagonist treatments given before embryo transfer in the women with endometriosis may maximize the chance of success of pregnancy outcomes.
Study design, size, duration: This longitudinal cohort study included 686 women with 1,205 embryo transfers during 2012-2017. The QoL of study women during IVF treatment was measured by FertiQoL comprising a Core module (mind/body, emotional, relational, and social domains) and a Treatment module (treatment environment and tolerability domains). The FertiQol scores were computed and scored in the range of 0-100, with higher scores indicating better QoL. The pregnancy outcomes of interest were chemical and ongoing pregnancies, and live birth.
Participants/materials, setting, methods: The QoL of study participants was assessed at the date before embryo transfer. Atosiban (a mixed oxytocin/vasopressin V1A receptor antagonist) can be administered intravenously during the embryo transfer. The GEE analysis was performed to assess the association between QoL during IVF treatment and subsequent pregnancy outcomes, with adjustment for time-varying factors (e.g., age) across multiple embryo transfers for an individual person. The analysis was further stratified by the number of embryo transfer cycle.
Main results and the role of chance: Compared to a control group of non-endometriosis women, endometriosis patients had significantly lower QoL, as indicated by the mind/body and treatment environment domain scores, total treatment scores, and total FertiQoL scores. FertiQoL scores were significantly associated with the success of pregnancy outcomes among non-endometriosis women; with a one unit increase in the emotional domain score, the probabilities of ongoing pregnancy and live birth significantly increased by 2.4% and 2.6%, respectively ($p < 0.05$). In overall study subjects (without stratified by the number of embryo transfer cycle), the use of atosiban was not significantly associated with positive pregnancy outcomes, while among those requiring more than two cycles, significantly increased pregnancy rates with atosiban use were found. These results regarding the association between atosiban use and IVF pregnancy outcomes are consistent in endometriosis and non-endometriosis groups.
Limitations, reasons for caution: This is a study enrolled the patients from a single medical center. Only the QoL from the date before ET was measured, while time-varying QoL data from different points of time during IVF treatment were not collected. The factors associated with the QoL of study patients were not further explored.
Wider implications of the findings: FeriQoL is useful in clinical practice to understand the QoL of women undergoing IVF treatment. Further developing clinical strategies to improve these patients' QoL would be of importance to enhance pregnancy outcomes. Oxytocin antagonists may enhance IVF pregnancy rates, especially among the patients requiring more than two embryo transfer cycles.
Trial registration number: nil

P-276 Resveratrol supplementation enhances decidualization of human endometrial stromal cells with concomitant decrease in cell proliferation

A.C. Mestre Citrinovitz[1], T. Strowitzki[1], A. Germeyer[1]

[1]Heidelberg University - Women's Hospital, Department of Gynecologic Endocrinology and Fertility Disorders, Heidelberg, Germany

Study question: Is resveratrol supplementation beneficial for human endometrial stromal cell *in vitro* decidualization?
Summary answer: Resveratrol supplementation enhances, in a dose-dependent manner, human endometrial stromal cell *in vitro* decidualization.
What is known already: Decidualization is the differentiation of endometrial stromal cells, a change needed to regulate trophoblast invasion and to support pregnancy. Decidualization follows endometrial stromal cell proliferation and it has been described that cell cycle arrest contributes to a proper decidualization. Interestingly, resveratrol, a natural compound derived from grapes with antioxidant properties, has been widely studied in relation to endometrial health. However, little is known about the effect of resveratrol supplementation during decidualization.
Study design, size, duration: This study was performed using primary human endometrial stromal cells (HESC). Endometrial biopsies from mid-late proliferative-phase were obtained from healthy women (34.4 +/- 1.1 years old) after written informed-consent that was obtained (approved protocol S-239/2005). HESC were decidualized *in vitro* with a decidualization cocktail containing medroxyprogesterone acetate, estradiol and 8-Bromo-cyclic adenosine monophosphate. Pre-decidualized cells (three days) were further treated with the decidualization cocktail supplemented with different doses of resveratrol (0 to 50 µM).
Participants/materials, setting, methods: Decidualization was evaluated by measuring prolactin (PRL) protein levels in cell culture supernatant and by measuring PRL and Insulin-like growth factor binding protein 1 (IGFBP1) mRNA levels by real time polymerase chain reaction (RT-PCR). Changes in the expression of genes related to cell cycle progression (KI67, CCNA1, CCNB1, CCND1, CDC2, CDK2, CDK4, p53, p21) and cell proliferation were evaluated for the

25 μM dose of resveratrol by RT-PCR and by crystal violet proliferation assay respectively.

Main results and the role of chance: Resveratrol supplementation increased, in a dose-dependent manner, the expression levels of PRL and IGFBP1, indicating an enhanced *in vitro* decidualization (PRL protein levels, mU/l: 0μM 193±35, 6.25μM 226±38, 12.5μM 270±49**, 25μM 296±61***, 50μM 274±49***; PRL mRNA levels, fold change related to 0μM of resveratrol: 0μM 1.0±0.0, 6.25μM 1.4±0.2*, 12.5μM 1.9±0.2***, 25μM 1.8±0.2***, 50μM 1.9±0.2***; IGFBP1 mRNA levels, fold change related to 0μM of resveratrol: 0μM 1.0±0.0, 6.25μM 1.4±0.2, 12.5μM 2.3±0.4, 25μM 2.4±0.6**, 50μM 1.3±0.2**). Enhanced decidualization was accompanied by a decrease in cell proliferation (25μM 4±1%* of reduced proliferation compared to 0μM) and by changes in the mRNA expression levels of key cell cycle regulators (mRNA levels; fold change related to 0μM of resveratrol: CCNB1 0.65±0.07***, CCND1 0.44±0.09**, CDK2 0.72±0.07**, CDK4 0.71±0.03***, p53 0.53±0.06***, p21 0.61±0.07***). Resveratrol supplementation seems to enhance decidualization by reinforcing the effect of the decidualization cocktail, in particular by reinforcing the inhibition in cell proliferation. Statistical differences *p<0.05 **p<0.01; ***p<0.001 compared to 0μM of resveratrol.

Limitations, reasons for caution: This study was performed *in vitro* using primary HESC. Further studies are necessary to confirm the effect of resveratrol during *in vivo* decidualization and to define the optimal dose and administration schedule for a beneficial outcome. Special caution needs to be taken in relation to resveratrol action over cell proliferation

Wider implications of the findings: Resveratrol seems to be an effective supplementation to reinforce hormone action during HESC decidualization. We believe that further insights into resveratrol action and its interaction with estradiol and progesterone signaling pathways could facilitate the identification of new therapeutic strategies for the improvement of women´s health.

Trial registration number: N/A

P-277 The Effect of Antibiotic Treatment on the Secretion of Proinflammatory Cytokines in Endometrial Stromal Cells in Patients with Chronic Endometritis

A. Nakamura[1], F. Kimura[1], J. Kitazawa[1], A. Morimune[1], A. Takashima[1], S. Kaku[1], T. Murakami[1]

[1]Gynecology, Obstetrics and Gynecology, Otsu, Japan

Study question: Can administration of oral antibiotics in patients with chronic endometritis (CE) reverse the increased inflammation observed in the endometrium?

Summary answer: The secretion of proinflammatory cytokines was reduced in the endometrial stromal cells of patients in whom CE was cured histologically but not in non-cured patients.

What is known already: CE is a mild, persistent inflammation of the endometrium, which is often asymptomatic but can cause infertility. CE is identified by the presence of plasma cells in the stromal layer of the endometrium. Plasma cell expression indicates the presence of a continuous immune response to any constituent of the endometrium. An increased pregnancy rate in CE patients treated with antibiotics has been reported. We have previously reported that CE patients had higher levels of inflammatory cytokines in their endometrial stromal cells. However, the effects on endometrial characteristics of curing CE with antibiotics are not yet known.

Study design, size, duration: Primary culture of endometrial stromal cells was performed. Thirty endometrial biopsied samples were obtained from 15 women from August 2018 to March 2019.

Participants/materials, setting, methods: The subjects were diagnosed with CE during in vitro fertilization (IVF) treatment. From each patient, endometrial biopsies were collected during the mid-secretory phase, both before and after antibiotic administration. Patients with low serum progesterone levels on the day of examination were excluded. The endometrial stromal cells from these samples were used for primary cell culture. To evaluate the status of inflammation, ELISA of the culture media was performed.

Main results and the role of chance: The numbers of patients with cured CE and persistent CE were 8 and 7, respectively.

For ELISA, endometrial stromal cells were cultured for 14 days and the supernatant was obtained. In the cured CE patients, secreted levels of TNF-α (p=0.0094), IL-1β (p=0.031), and IFN-γ (p=0.0089) were significantly decreased by the antibiotic treatment, whereas the concentration of IL-6 was not significantly altered. In the samples obtained after antibiotic treatment, secreted levels of TNF-α (p=0.024), IL-1β (p=0.019), and IFN-γ (p=0.0071) were significantly lower in the patients in whom CE was cured than in the persistent CE patients.

Limitations, reasons for caution: The number of samples was small. However, there were still significant differences in TNF-α, IL-1β, and IFN-γ levels. We speculate that with an increased number of cases, a significant difference in IL-6 may be observed.

Wider implications of the findings: When CE was cured, secretion of proinflammatory cytokines from endometrial stromal cells was normalized to the level of healthy patients. Thus, CE was related to the uterine microbiome because antibiotic treatment was effective in suppressing proinflammatory cytokine secretion from endometrial stromal cells.

Trial registration number: not applicable

P-278 Vitamin D as an effective treatment to reduce cell proliferation and extracellular matrix formation in human uterine leiomyomas regardless MED12 mutation status

A. Corachán[1], M.G. Trejo[1], M.C. Carbajo-García[1,2], A. Faus[1], J. Escrig[3], J. Monleón[3], A. Pellicer[1,4], I. Cervelló[1], H. Ferrero[1]

[1]IVI Foundation- IIS La Fe, Innovation, Valencia, Spain ;
[2]Universidad de Valencia, Pediatrics- Obstetrics and Gynecology, Valencia, Spain ;
[3]Hospital Universitario y Politécnico La Fe, Obstetrics and Gynaecology Service, Valencia, Spain ;
[4]IVIRMA Rome, Department of Reproductive Endocrinology and Infertility, Rome, Italy

Study question: Could Vitamin D be an effective treatment to reduce the size of different molecular subtypes of human uterine leiomyoma?

Summary answer: Vitamin D inhibited cell proliferation, Wnt/β-catenin and TGFβ pathways in human uterine leiomyoma primary cells from two molecular subtypes: *MED12*-mutated and wild type.

What is known already: Uterine leiomyomas, the most common benign tumor of reproductive tract, are clinically and scientifically treated as a single entity despite the existence of four molecular subtypes, among which *MED12* mutation is the most frequent genetic alteration. It has been suggested that Wnt/β-catenin and TGFβ pathways contribute to *MED12*-mutated leiomyoma growth via cell proliferation and extracellular matrix (ECM) formation stimulation. In pursuit of new treatments, several studies propose Vitamin D (VitD) as a safe therapeutic option to reduce leiomyomas. However, these studies do not examine the existing genetic heterogeneity, a crucial fact to consider in the development of an efficient treatment.

Study design, size, duration: Prospective study in which human uterine leiomyomas (UL) and matched myometrium (M) tissues (n=37) were analyzed to identify *MED12* mutations. The status of Wnt/β-catenin and TGFβ pathways was determined in a paired design study in UL and M tissues depending on the presence/absence of *MED12* mutation. Similarly, the effect of VitD treatment on proliferation and the above-mentioned pathways was evaluated *in vitro* in human uterine leiomyoma primary (HULP) cells depending on *MED12* status (n=3/group).

Participants/materials, setting, methods: UL and M were collected from 37 patients (30-61 years) and *MED12* mutations were identified by Sanger sequencing in both tissues. The expression level of *WNT4, β-Catenin* (Wnt/β-catenin pathway), *TGFβ3, MMP9* (TGFβ pathway) in UL and M was determined by quantitative PCR (qPCR). HULP cells were isolated and treated *in vitro* with/without 1000nm VitD (48h). To evaluate treatment effect, the expression of the above-mentioned genes along with *PCNA* (cell proliferation marker) was measured by qPCR.

Main results and the role of chance: Sequencing data showed that 46% of UL presented *MED12* mutations, while no mutations were detected in their corresponding M. According to this, UL were divided into two groups depending on *MED12* status: *MED12*-mutated or wild type (WT). The evaluation of Wnt/β-catenin and TGFβ pathways in UL and M tissues showed that *MED12*-mutated UL presented significant higher levels of *WNT4* (fold regulation [fr]= 13.05, p=0.02), *β-Catenin* (fr= 2.84, p=0.01), *TGFβ3* (fr= 5.27, p=0.002) and *MMP9* (fr=13.73,p=0.004) than their corresponding M, while no significant differences were found in WT UL. *In vitro* study demonstrated that VitD decreased the expression of the cell proliferation marker *PCNA* in HULP cells from

MED12-mutated (fr= -1.7, p=0.05) and WT UL (fr= -2.8, p=0.01). Likewise, the analysis of TGFβ pathway genes showed that VitD significantly inhibited *TGFβ3* expression in HULP cells from *MED12*-mutated (fr= -3.1, p=0.02) and WT UL (fr= -4, p=0.02). Accordingly, *MMP9* expression decreased after VitD treatment in HULP cells from both *MED12*-mutated and WT UL (fr=-1.47 and -1.45, respectively). Regarding Wnt/β-catenin pathway, the expression of *β-Catenin* was decreased in HULP cells from both *MED12*-mutated and WT UL (fr= -1.2 and -1.5, respectively) after VitD treatment.

Limitations, reasons for caution: Considering the great variability that exists between patients, the limitation of this work includes the small sample size in the *in vitro* study. Further studies are necessary to translate *in vitro* results into practical clinical applications.

Wider implications of the findings: Notwithstanding the molecular differences found between *MED12*-mutated and WT UL, VitD could inhibit Wnt/β-catenin and TGFβ pathways in all HULP cells. These results suggest that VitD would be an effective treatment to reduce tumor size via the reduction of cell proliferation and ECM formation in different molecular subtypes of UL.

Trial registration number: not applicable

P-279 Aberrant expression of immune cells and Notch1 activation coincides with epithelial to mesenchymal transition in the development of adenomyosis in mice.

M. Bourdon[1], P. Santulli[1], L. Doridot[2], M. Jeljeli[3], C. Nicco[2], S. Chouzenoux[2], C. Chène[2], F. Batteux[3], C. Chapron[1]

[1]université Paris Centre, Gynecology and reproductive médecine, Paris, France ; [2]INSERM, Institut Cochin, Paris, France ; [3]Université Paris Centre, Immunology, Paris, France

Study question: In a mouse model of adenomyosis, do innate and adaptive immune cells and Notch1 pathway have any role in the development of adenomyosis?

Summary answer: Notch1 activation and aberrant expression of innate and adaptive immune cells promote adenomyosis developpement through the epithelial to mesenchymal transition (EMT).

What is known already: EMT has been implicated in the development of adenomyosis, along with a dysregulated innate and adaptive immune response. One hypothesis is that immune changes in uterus may promote endometrial cells proliferation and migration to myometrium and EMT activation, leading to adenomyosis lesions. Besides, inflammation is known to be a potential activator of the Notch signaling pathways known to have capacity to favor the EMT process.

Study design, size, duration: Adenomyosis was induced in 26 female CD1 mice by neonatal dosing of tamoxifen. Another 28 were neonatally dosed without tamoxifen (control group). These mice were sacrificed serially and their tissue samples were subsequently evaluated.

Participants/materials, setting, methods: Female CD-1mice with and without induced adenomyosis were sacrificed in batch at 30,60 and 90 days of age. The depth of myometrial infiltration of endometrial tissues was assessed with histology. Immune cells markers disturbance (CD45,Ly6C1,CD86,Arginine1,CD19,CD4, CD8) were analysed by RTqPCR. Notch1 and target genes (HEY1,HEY2,HES1,HES5) were analysed by RTqPCR. The Notch intracellular domain (NICD) was measured with western blot analysis. Analysis of EMT biomarkers was performed with RTqPCR (E-Cadherin,Vimentin,TGFb,SNAIL1,SLUG,Snail 3) and Western blot (E-Cadherin,Vimentin).

Main results and the role of chance: Aberrant expression of immune cells markers was observed in uteri from adenomyosis -mice over the development of the disease. Expression of innate inflammatory cells markers, notably M1 macrophages and natural killer's cells were increased from an early stage (Day30) compared to uteri from control mice, followed by an increase of CD4 T cells at day 60. Inversely, expression of CD19 B cells were significantly decreased during all studied stage. Activated-Notch1 was also highly activated during adenomyosis development compared to control mice at day 30 and D60. Concomitantly, some markers for EMT also was increased. This Notch activation could be related to the activation of EMT and migration of endometrial cells within the myometrium.

Limitations, reasons for caution: This study is limited by the use of an animal model and the lack of intervention.

Wider implications of the findings: This study provides evidence that adenomyotic lesions could be a consequence of immune modifications and Notch1 activation. Hypothesis is that an increase in local inflammation lead to activation of Notch1pathway. NICD, induced in the nucleus, transcriptional activation of genes related to EMT leading to migration of endometrial cells through myometrium.

Trial registration number: NA

P-280 Relation between clinical profiles and adenomyosis phenotypes assessed by magnetic resonance imaging

J. Oliveira[1], M. Bourdon[1], L. Marcellin[1], C. Bordonne[1], L. Maitrot Mantelet[1], A.E. Millischer[2], G. Plu Bureau[1], P. Santulli[1], C. Chapron[1]

[1]University of Paris.- Faculty of Medicine-, Department of Obstetrics and Gynecology II and Reproductive Medicine- Cochin University Hospital., Paris, France ; [2]Centre de Radiologie Bachaumont, Centre de diagnostique médicale de Paris IMPC, Paris, France

Study question: Do adenomyosis phenotypes such as focal (in the outer myometrium) and diffuse adenomyosis, as diagnosed by magnetic resonance imaging (MRI), have the same clinical characteristics?

Summary answer: Focal and diffuse adenomyosis phenotypes exhibit distinct clinical profiles in terms of both women's characteristics (age, gravity, previous surgery, endometriosis) and symptoms (menorrhagia, infertility).

What is known already: Uterine adenomyosis is characterized by the presence of endometrial glands and stroma deep within the myometrium. Various forms have been described, including focal adenomyosis(FOC-ADE), which corresponds to nodular lesions separated from the junctional zone in the outer myometrium, and diffuse adenomyosis (DIF-ADE), which is characterized by endometrial implants scattered throughout the myometrium and enlargement of the junctional zone. Although the pathogenesis of adenomyosis is not clearly understood, several lines of evidence suggest that these two phenotypes could have distinct origins. The clinical presentation of different forms of adenomyosis in patients warrants further investigation.

Study design, size, duration: This was an observational study. Non-pregnant patients, aged between 18 and 42 years, who had undergone surgical exploration for benign gynecological conditions at our institution between May 2005 and May 2018 and with a preoperative uterine MRI were included in the study. Data was prospectively collected by a standardized questionnaire regarding women's histories and clinical symptoms, completed during a face-to-face interview conducted by the surgeon in the month preceding the surgery.

Participants/materials, setting, methods: 248 women had a radiological diagnosis of adenomyosis assessed by MRI and signed informed consent. Based on the MRI findings, the women were diagnosed as having FOC- and/or DIF-adenomyosis. The women were allocated to two groups according to the adenomyosis phenotype (FOC-ADE versus DIF-ADE). Women exhibiting an association of both the FOC- and the DIF-adenomyosis form were excluded.

Main results and the role of chance: All up, following the MRI findings, 109 women (44.0%) exhibited only FOC-ADE and 78 (31.5%) had only DIF-ADE. The women with FOC-ADE were significantly younger (mean of31.9 years of age ± 4.6 SD versus 33.8 ± 5.2 SD, respectively; p = 0.006), more often nulligravid (74.3%versus 33.3%; p ≤ 0.001), exhibited a higher rate of associated-endometriosis (p= 0.001) especially deep infiltrating endometriosis (89.0% versus 30.8%) and a higher rate of infertility (p=0.021) especially primary infertility (25.1% versus 9.0%) compared to the women in the DIF-ADE group. Moreover, the women exhibiting DIF-ADE had significantly more often a history of previous uterine surgery (21.8% versus 6.4%;p = 0.002) and menorrhagia (79.5% versus 53.2%; p= 0.001) compared to the women with FOC-ADE. No differences in the pain scores (i.e., dysmenorrhea, non-cyclic pelvic pain, and dyspareunia) were observed between the two groups.

Limitations, reasons for caution: The exclusive inclusion of surgical patients as the limitation age could constitute possible selection bias. Moreover, the women referred to our center may have suffered from particularly severe clinical symptoms.

Wider implications of the findings: In clinical practice, these two forms of adenomyosis should be differentiated using an appropriate imaging work-up in order to search for associated endometriosis and to devise a tailored therapeutic strategy. To better understand the pathogenesis, future mechanistic studies aimed at better characterization of diffuse and focal adenomyosis are needed.

Trial registration number: not applicable

P-281 Regulatory effect of prohibitin on glucose metabolism of granulosa cell in endometriosis.

M. Jingqin[1], C.Y. Zhong[1], L. Gao[1], J. Zhang[1], J.Y. Zhang[1], L. Dai[1], Y.D. Mao[1]

[1]First Affiliated Hospital of Nanjing Medical University, Clinical Center of Reproductive Medicine, Nanjing, China

Study question: What role does prohibitin play in glucose metabolism in granulosa cells of patients with endometriosis?
Summary answer: Patients with endometriosis shows the high expression of prohibitin to make up for glucose aerobic metabolism defects.
What is known already: The metabolic dysfunction in granulosa cells of endometriosis patients has been examined by various groups. Prohibitin is ubiquitously expressed, mainly presenting in mitochondria to maintain the membrane potential of mitochondrial, to facilitate in assembly of respiration chain. Meanwhile, prohibitin play a critical role in energy metabolism.
Study design, size, duration: In this study, glucose metabolic phenotype of granulosa cells were examined in vitro. The human granulosa cell line KGN were transfected with lentivirus to knock down and over express the expression of prohibitin. Afterwards, the glucose metabolism phenotype and cell vitality were evaluated.
Participants/materials, setting, methods: The primary granulosa cells were collected to test the glucose metabolism features as follows: lactate production (Lactate assay kit) glucose consumption (Glucose assay kit) and the expression of prohibitin and glucose metabolism related enzymes(q-PCR and Western blotting). The KGN was transfected with lentivirus to knock down and over express the expression of prohibitin. The mitochondrial function and cell proliferation were tested by ATP assay kit and CCK8 kit.
Main results and the role of chance: In women with endometriosis, significantly increased glucose consumption, lactate production, prohibitin expression and aberrant expression of glycolysis-related enzymes were found compared with women who do not have endometriosis ($P < 0.05$). To determine whether prohibitin regulated glucose metabolism by affecting the expression of glycolysis related enzymes, the expression of enzymes were evaluated after lentivirus transfection in KGN. When prohibitin was down regulated in KGN, the mRNA expression of HK1, ENO1α, PDHA, CS, SDHB and ATP5A were significantly decreased ($P < 0.05$). When prohibitin was over expressed, the the mRNA expression of Glut1, HK1, ENO1α, LDHA and PDHA were significantly increased ($P < 0.05$). Meanwhile, the over-expression of prohibitin led to elevated glucose consumption and lactate production ($P < 0.05$), whereas, the knock-down of prohibitin made no change. Additionally, cell viability and proliferation were significantly inhibited after the down regulation of prohibitin($P < 0.05$).
Limitations, reasons for caution: More experiments are necessary to explore the specific molecule signal pathway involved with prohibitin to regulate the glucose metabolism.
Wider implications of the findings: Women with endometriosis exhibits aberrant glucose metabolism and high level expression of prohibitin in granulosa cells. According the results, prohibitin may improve the glycolysis to make up the energy output deficiency resulting from mitochondrial dysfunction, to exert the protective effect on gruanlosa cells in women with endometriosis.
Trial registration number: 81671438, 81730041

P-282 Rectal water-contrast transvaginal ultrasonography versus sonovaginography for the diagnosis of posterior deep pelvic endometriosis: a prospective comparative study

F. Barra[1], C. Scala[2], V.G. Vellone[3], C. Stabilini[3], S. Ferrero[1]

[1]IRCCS Ospedale Policlinico San Martino- University of Genoa, Academic Unit of Obstetrics and Gynecology, Genoa, Italy ;
[2]Gaslini Institute, Unit of Obstetrics and Gynecology, Genoa, Italy ;
[3]IRCCS Ospedale Policlinico San Martino- University of Genoa, Department of Surgical and Diagnostic Sciences DISC, Genoa, Italy

Study question: To investigate the accuracy of rectal water-contrast transvaginal ultrasonography (RWC-TVS), and sonovaginography (SVG) in patients with clinical suspicion of posterior deep infiltrating endometriosis (DIE).
Summary answer: RWC-TVS and SGV have similar accuracy in the diagnosis of posterior DIE. However, RWC-TVS is more accurate in diagnosing presence and characteristics of rectosigmoid endometriosis.
What is known already: Surgical treatment of posterior DIE may be challenging for surgeons and carry significant risks for patients. Preoperative assessment by imaging of the location, characteristics and presence of posterior DIE nodules is important in order to inform the patient on the possible treatments and to provide informed consent to patients undergoing surgery. RWC-TVS and SGV consist of enhanced transvaginal ultrasound scans based on the introduction of contrast into the rectum and vagina, respectively.
Study design, size, duration: This was a prospective comparative study, enrolling 221 women with clinical suspicion of posterior DIE.
Participants/materials, setting, methods: Patients with previous diagnosis of posterior DIE by magnetic resonance, computed tomography or laparoscopy were excluded from the study. All patients underwent RWC-TVS and SVG, performed by two independent ultrasonographers, informed of the patients' clinical history and symptoms but blinded to the other diagnostic exam. The presence of rectovaginal septum, rectosigmoid, uterosacral ligaments, and vagina endometriosis was investigated. Patients underwent laparoscopy within the following 6 months. Imaging findings were compared with surgical and histological results.
Main results and the role of chance: In 89.6% (n=198/221) of the patients, posterior DIE was laparoscopically confirmed. The nodules were localized in rectovaginal septum (36.4%), rectosigmoid (24.2%), uterosacral ligaments (66.1%), and vagina (6.4%). There was no significant difference in the performance of RWC-TVS and SVG in diagnosing posterior DIE (p=0.187). The accuracy of RWC-TVS in the detection of posterior DIE was 92.2%, sensitivity was 89.4%, specificity 95.0%, positive predictive value 94.6%, negative predictive value 90.1%, positive likelihood ratio 18.0 and negative likelihood ratio 0.1. For SVG, the corresponding values were 88.8%, 82.8%, 94.9%, 94.3%, 84.4%, 16.3 and 0.2, respectively. RWC-TVS had higher accuracy than SVG in diagnosing presence of rectosigmoid endometriosis (sensitivity 91.2% vs 80.7%; specificity 95.8% vs 89.0%; p<0.001), depth of penetration of endometriosis in the intestinal muscularis propria (89.3% vs 79.5%; 90.2% vs 77.8%; p<0.001), and distance between the nodule and anal verge (91.3% vs 77.2%; 88.3% vs 74.2%; p<0.001). There was no significant difference between the two techniques in diagnosing endometriotic nodules of utero-sacral ligaments (83.5% vs 82.1%, 82.4% vs 79.1%; p=0.312) and rectovaginal septum (92.5% vs 88.2%, 87.8% vs 85.3%; p=0.431). RWC-TVS had lower sensitivity (75.0% vs 91.7%, p=0.031) but similar specificity (98.1% vs 97.6%; p=0.453) than SVG in diagnosing vaginal endometriosis.
Limitations, reasons for caution: A limitation of the study if that the diagnostic performance of both techniques is depending on the experience of the examiners. The surgeons were aware of the findings of these diagnostic exams. Both techniques cannot diagnose endometriotic nodules located above the rectosigmoid.
Wider implications of the findings: This study showed that RWC-TVS and SVG have similar performance in diagnosing deep posterior DIE. RWC-TVS is more accurate that SVG in assessing the presence and characteristics of rectosigmoid endometriosis.
Trial registration number: Not applicable

P-283 Three-dimensional rectal water contrast transvaginal ultrasonography versus virtual colonoscopy for diagnosing presence and characteristics of rectosigmoid endometriosis

F. Barra[1], E. Biscaldi[2], C. Scala[3], V.G. Vellone[4], C. Stabilini[4], S. Ferrero[1]

[1]IRCCS Ospedale Policlinico San Martino - University of Genoa, Academic Unit of Obstetrics and Gynecology, Genoa, Italy ;
[2]Galliera Hospital, Radiology, genova, Italy ;
[3]Gaslini Institute, Unit of Obstetrics and Gynecology, Genoa, Italy ;
[4]IRCCS Ospedale Policlinico San Martino - University of Genoa, Department of Surgical and Diagnostic Sciences DISC, Genoa, Italy

Study question: To compare the performance of three-dimensional rectal water contrast transvaginal ultrasonography (3D-RWC-TVS) and computed tomographic colonography (CTC) in predicting the presence and characteristics of rectosigmoid endometriosis.

Summary answer: 3D-RWC-TVS and CTC have similar diagnostic accuracy for diagnosing rectosigmoid endometriosis. However, CTC is more accurate in ruling out the presence of sigmoid endometriotic nodules.
What is known already: A non-invasive diagnosis of bowel endometriosis is relevant to provide the patients information on the potential treatments (hormonal therapies or surgery) and to obtain adequate informed consent in case of surgery. Over the last 10 years, RWC-TVS and CTC based on the distention of the rectosigmoid have been proposed with the aim to improve the diagnosis of deep infiltrating endometriosis. Both exams allow the evaluation of bowel using a pseudoendoscopic view.
Study design, size, duration: This was a prospective comparative study including patients who were referred to our institution for suspicion of rectosigmoid endometriosis between January 2018 to December 2019.
Participants/materials, setting, methods: Patients with symptoms suggestive of rectosigmoid involvement by endometriosis were included in the study. They underwent 3D-RWC-TVS performed by a sonographer skilled in the diagnosis of deep endometriosis. CTC was done within the following three months by a radiologist expert in the diagnosis of deep endometriosis, blinded to the results of the previous ultrasonographic exam. Patients underwent surgery within the following six months. Imaging findings were compared with surgical and pathologic results.
Main results and the role of chance: Out of 68 women included, 37 (48.9; 95% C.I. 38.2%-59.7%) had rectosigmoid nodules which required bowel surgery. The main nodules were located on the sigmoid in 16 (43.2%) patients, on the rectosigmoid junction in 4 (10.8%) patients, on the upper rectum in 10 (27.0%) patients and on the lower rectum in 7 patients (18.9%). There was no significant difference in the accuracy of 3D-RWC-TVS and CTC in diagnosing the presence of rectosigmoid endometriotic nodules (p = 0.118). However, a subgroup analysis demonstrated that CTC was more precise than 3D-RWC-TVS in diagnosing endometriosis located in sigmoid (p = 0.016). The presence of an endometrioma with diameter > 4 cm was positively statistically correlated to lack of identification of sigmoid endometriotic nodules (phi coefficient 0.516; p=0.039) by performing 3D-RWC-TVS. Both exams estimated similarly the largest diameter of the main endometriotic nodule, independently of their location (p = 0.090). CTC was more accurate than 3D-RWC-TVS in estimating the distance between the lower margin of the intestinal nodule and the anal verge (p = 0.030). There was no significant difference in the performance of 3D-RWC-TVS and CTC in diagnosing multifocal disease (p = 1.000).
Limitations, reasons for caution: A limitation of both techniques is represented by the extensive experience of the radiologist and the gynecologist performing CTC and 3D-RWC-TVS, respectively, which may have influenced the performance of these techniques in ruling out the presence of rectosigmoid endometriosis.
Wider implications of the findings: 3D-RWC-TVS and CTC have similar diagnostic performance for diagnosing rectosigmoid endometriosis, although 3D-RWC-TVS is less accurate in detecting sigmoid nodules. CTC may be combined with 3D-RWC-TVS because of the high diagnostic performance in detecting rectosigmoid endometriosis and the ability to diagnose endometriotic nodules located above the rectosigmoid junction.
Trial registration number: Not applicable

P-284 In silico screening and variant analysis in the transcript profile of eutopic endometrium from infertile women with endometriosis and controls during the implantation window

M. Da Broi[1], J. Plaça[2], R. Ferriani[1], P. Navarro[1]

[1]Ribeirão Preto School of Medicine- University of São Paulo, Department of Obstetrics and Gynecology, Ribeirão Preto, Brazil ;
[2]Center for Medical Genomics- HCFMRP/USP, Department of Genetics, Ribeirão Preto, Brazil

Study question: Is there a pattern of functional mutations in transcripts of the eutopic endometrium of infertile women with endometriosis during the implantation window that could be related to impaired endometrial receptivity?
Summary answer: No pattern of functional mutations was identified in the transcripts of the eutopic endometrium of infertile women with endometriosis during the implantation window.
What is known already: The molecular and functional impairment of the eutopic endometrium in women with endometriosis is questioned as a possible mechanism involved in the disease-related infertility. Recently, the sequencing of mRNAs (RNA-Seq) did not show differential expression in the transcripts of infertile patients with endometriosis when compared to infertile and fertile controls during the implantation window. However, these findings do not exclude the possibility of existing other molecular alterations with possible impact on protein synthesis and, potentially, on the establishment of endometrial receptivity.
Study design, size, duration: In this descriptive study, data from RNA-Seq analysis of endometrial biopsies collected during the implantation window from 17 patients (3 infertile women with endometriosis I/II, 3 infertile women with endometriosis III/IV, 6 infertile controls and 5 fertile controls) were *in silico* analyzed by bioinformatics tools for variant discovery, functional mutations identification and selection of proteins possibly affected. A targeted study of the alterations found was performed to understand the data in the disease's context.
Participants/materials, setting, methods: Analyzes were performed in the GATK (Genome Analysis Toolkit), following the best practices recommended for the discovery of variants in RNA-Seq data. Data were filtered according to the coverage, gene region, variant function and description in NCBI databases. Subsequently, the variants were evaluated by Pathogenicity Predictors. The first predictor applied was the CADD, followed by the agreement of the predictors PROVEAN, SIFT and Polyphen 2 in classifying deleterious or possibly deleterious variants.
Main results and the role of chance: None of the variants found were common to other samples within the same group, as well, no mutation was repeated in patients with endometriosis, infertile controls and fertile controls. In the endometriosis group, 9 mutations predicted to be harmful were identified. Only one of the mutations (in the gene *SLC2A9*) had already been described as associated with clinical conditions (familial renal hypouricemia), with no evident impact on the endometrium. However, when crossing the genes related to the 9 mutations found with the keywords "endometriosis" and/or "endometrium", the gene *CMKLR1* was associated either with the inflammatory response in endometriosis or with important endometrial processes for pregnancy establishment.
Limitations, reasons for caution: The small sample size does not allow us to state whether there are differential mutations among women with endometriosis compared to fertile and infertile controls. Moreover, the search for variants was performed on RNA-Seq data and only expressed transcripts were evaluated. Regulatory regions were not analyzed.
Wider implications of the findings: Despite no pattern of mutation was found, we ponder the small sample size and the analysis on RNA-Seq data. Considering the study's purpose of screening and the *CMKLR1* importance on endometrial modulation, it could be a candidate gene for further studies evaluating mutations in eutopic endometrium from endometriosis patients.
Trial registration number: Not applicable

P-285 Pretreatment with dienogest in women with endometriosis undergoing in vitro-fertilization after a previous failed cycle

F. Barra[1], C. Scala[2], G. Evangelisti[1], S. Ferrero[1]

[1]IRCCS Ospedale Policlinico San Martino - University of Genoa, Academic Unit of Obstetrics and Gynecology, Genoa, Italy ;
[2]Gaslini Hospital, Unit of Obstetrics and Gynaecology, Genoa, Italy

Study question: To investigate the effect of dienogest (DNG) administered in women with endometriosis undergoing IVF after a previous failed cycle.
Summary answer: In women with endometriosis, particularly in those with large endometriomas, implantation rate and clinical pregnancy rate can be improved by pretreatment with DNG.
What is known already: It is generally assumed that diminished ovarian reserve, impaired endometrial receptivity and low quality of embryos may be causes of the lower *in vitro* fertilization (IVF) outcomes of women with endometriosis. The use of prolonged courses of hormone therapy may improve IVF outcome of patients with endometriosis.
Study design, size, duration: This was a retrospective analysis of a prospective database collected between January 2016 to July 2019. Women who failed a previous IVF cycle and all subsequent embryo transfers, with concomitant diagnosis of endometriosis at transvaginal ultrasonography or magnetic resonance imaging were included in this study.
Participants/materials, setting, methods: Inclusion criteria were age ≤40 years; basal FSH <14.0 IU/L and anti-Müllerian hormone (AMH) blood level

≥0.5 g/mL. Women who underwent previous surgical treatment of endometriosis were excluded. Following a failed IVF cycle, patients either directly underwent IVF without receiving hormonal treatment or received a three-month DNG (2 mg daily) treatment before IVF. The choice of treatment was based on patients' preference.

Main results and the role of chance: 151 patients were included in the study, 88 (58.3%) underwent IVF without previous hormonal treatment and 63 (41.7%) received pretreatment with DNG. At baseline, there was no significant difference in age, average duration of infertility, antral follicle count, basal serum FSH, AMH and presence of endometriomas between the two groups. The implantation rate and clinical pregnancy rate were significantly higher in the DNG-treated group (39.7% and 33.3%) compared with the non-treated group (23.8% and 18.2%; p=0.037 and p=0.033, respectively). The largest diameter and volume of endometriomas significantly decreased after pretreatment with DNG (-0.7 ± 0.8 cm and -3.1 ± 7.7 cm^3, p<0.001 and p<0.001, respectively). A subgroup analysis demonstrated that the use of DNG increased significantly the number of oocytes retrieved (6.1 ± 2.8 versus 5.3 ± 2.2, p=0.039), of 2 pronuclear (2PN) embryos (2.9 ± 1.6 versus 2.2 ± 1.8, p=0.015) and of blastocysts (2.4 ± 1.6 versus 2.0 ± 1.4, p=0.049) obtained in patients with endometriomas with diameter ≥ 4 cm.

Limitations, reasons for caution: While baseline demographics and first IVF cycle stimulation parameters for the studied patients were comparable, the non-randomized allocation of subjects may mask a hidden bias in this study.

Wider implications of the findings: IVF outcomes of women with endometriosis seem to be improved after pretreatment with DNG. In particular, this progestin may give a benefit in patients with large endometriomas.

Trial registration number: Not applicable

P-286 Dynamic properties of endometrial macrophages regulate tissue homeostasis and disease in the uterus

E. Mann[1], N. Scott[1], L. Lacerda Mariano[2], P. Ruane[3], M. Ingersoll[2], L. Mohiyiddeen[4]

[1]University of Manchester, Lydia Becker Institute of Immunology and Inflammation, Manchester, United Kingdom ;
[2]Institut Pasteur, Immunology, Paris, France ;
[3]University of Manchester, Maternal and Fetal Health Research Centre, Manchester, United Kingdom ;
[4]Manchester NHS Foundation Trust, Reproductive Medicine, Manchester, United Kingdom

Study question: Do macrophages contribute to tissue remodelling and repair in the uterus, and contribute to pathology in fibrosis/Asherman's syndrome when their function is dysregulated?

Summary answer: Monocytes recruited from the bloodstream generate uterine macrophages with distinct roles in tissue repair and remodelling, and macrophages may be dysregulated in Asherman's syndrome.

What is known already: Dysregulated tissue degradation, repair and remodelling and can lead to fibrosis and adhesions in the uterus (known as Asherman's syndrome), causing reproductive dysfunction. In other tissues, macrophages (immune cells) are central to tissue remodelling and repair. However, the ontogeny and functions of macrophages within the uterus are poorly defined, despite dysregulation of macrophage recruitment and function driving fibrosis at other mucosal sites.

Study design, size, duration: Macrophages were characterized at different stages of the estrous cycle in mice (proestrus, estrus, metestrus and diestrus), which reflects the hormone fluctuations that regulate tissue remodelling and receptivity to embryo implantation occurring in the human uterus (n=10-15 per estrous cycle stage). Differentiation of murine endometrial macrophages from infiltrating blood monocytes *in vivo* was assessed using methods described below (n=6-12 per time point). Macrophages were characterized from human endometrial biopsies, obtained following informed consent (n=5).

Participants/materials, setting, methods: Macrophages were characterised from uterine tissue (mouse and human) by multi-parameter flow cytometry following enzymatic digestion. Mice were staged using vaginal smear cytology to determine estrous cycle phases according to proportions of epithelial cells, cornified cells and leucocytes. Ontogeny of murine endometrial macrophages was defined at multiple time points 8 and 20 weeks after reconstitution of irradiated mice (uterus lead shielded) with congenic bone marrow to allow tracking of donor cells in uterine tissue.

Main results and the role of chance: Mature macrophages were present at all stages of the murine estrous cycle and expressed features indicating involvement in tissue remodelling and repair, including high levels of the IL-4 receptor, CSF-1 receptor, mannose receptor CD206 and protein Relmα (critical for remodelling and repair in other tissues). Accordingly, endometrial macrophages were more responsive to IL-4 than mucosal macrophages from other tissues (p<0.001). Unlike other tissue macrophages, endometrial macrophages did not proliferate *in situ* but the substantial majority were differentiated from circulating monocytes recruited into the endometrium in a manner dependent on the chemokine receptor CCR2 (p<0.01). By 20 weeks post irradiation, turnover of macrophages in the uterus from blood monocytes was higher than in the intestine (p<0.05), previously demonstrated to have one of the highest rates of replenishment. Numbers of monocytes in the uterus were profoundly increased during estrus (receptivity period when estrogen is low; p<0.01), likely accounting for the high rate of turnover into macrophages. These recruited macrophages were highly responsive to IL-4 and exhibited enhanced levels of CD206 and Relmα compared to their tissue-resident counterparts (p<0.001). Preliminary data suggest human endometrial macrophages are drastically expanded in Asherman's syndrome and that these cells have immature properties suggesting dysregulated differentiation.

Limitations, reasons for caution: The remodelling events that occur throughout the murine estrous cycle differ from those in the human menstrual cycle in terms of endometrial shedding/menstruation which does not occur in mice. Nonetheless, mouse models provide the opportunity to perform the *in vivo* ontogeny and functional studies that are not possible in humans.

Wider implications of the findings: The estrous cycle is marked by dynamic changes in populations of endometrial monocytes/macrophages that are likely to play a key role in tissue remodelling during the receptivity period in which implantation can occur in homeostasis, but may contribute to pathology in fibrotic disease such as Asherman's syndrome when dysregulated.

Trial registration number: not applicable

P-287 Suppression of ovarian activity during co-administration of the oral gonadotropin-releasing hormone receptor antagonist relugolix, estradiol, and norethindrone acetate in healthy female volunteers

I. Duijkers[1], C. Klipping[1], C. Draeger[2], B. Schug[3], R.S. Wedemeyer[3], J. Li[4], J.C. Arjona Ferreira[4], E. Migoya[4]

[1]dinox consultancy, Groningen, The Netherlands ;
[2]dinox GmbH, Berlin, Germany ;
[3]SocraTec R&D GmbH, Oberursel, Germany ;
[4]Myovant Sciences Inc., Brisbane, U.S.A.

Study question: Does relugolix combination therapy (relugolix, oral gonadotropin-releasing hormone (GnRH) receptor antagonist, 40mg, estradiol (E_2) 1mg and norethindrone acetate (NETA) 0.5mg) suppress ovarian activity and ovulation?

Summary answer: Once daily oral administration of relugolix combination therapy for 84 days suppressed ovarian activity and inhibited ovulation in 100% of subjects.

What is known already: Relugolix competitively binds to the GnRH receptor, preventing the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), thereby decreasing the production of E_2 and progesterone (P). By lowering systemic concentrations of E_2 and P, oral administration of 40 mg relugolix monotherapy is effective in the treatment of symptoms associated with uterine fibroids, however, is associated with hypoestrogenic symptoms and bone mineral density loss. In current phase 3 trials in patients with uterine fibroids or endometriosis, relugolix treatment is combined with 1 mg E_2 and 0.5 mg NETA to mitigate hypoestrogenic side effects.

Study design, size, duration: An open-label, single treatment-group clinical study in 70 women, 67 of which completed the study. The study included a pre-treatment cycle to assess ovulatory status, an 84-day treatment period to assess ovarian activity during combination treatment (co-administration of a relugolix 40mg tablet and an E2/NETA 1mg/0.5mg tablet (Activelle®)), and a post-treatment cycle to assess return of ovulation. Subjects visited the study center every third (± 1) day during the entire study.

Participants/materials, setting, methods: The study, conducted in a clinical research center, included healthy premenopausal women 18-35 years of age. During the study visits ultrasound measurements of follicular growth and endometrial thickness, and blood sampling for determination of serum E_2, P, FSH and LH concentrations were performed. Ovarian activity was categorized using the Hoogland-Skouby score. Safety parameters and vaginal bleeding were also assessed. The primary endpoint was the proportion of subjects with Hoogland-Skouby scores <5 during the treatment period.

Main results and the role of chance: Once-daily dosing with relugolix combination therapy resulted in suppression of ovarian activity and inhibition of ovulation in 100% of women as demonstrated by Hoogland-Skouby scores <5 during the entire 84-day treatment period. In most subjects the largest follicular diameter remained below 10 mm throughout treatment, resulting in a Hoogland-Skouby score of 1 (no ovarian activity). Mean and median E2 concentrations, reflecting both endogenous and exogenously-administered E_2, were between 110 and 160 pmol/L (30 and 44 pg/mL). Serum P, FSH, and LH concentrations were suppressed for the duration of treatment. Ovarian activity resumed after treatment discontinuation with ovulation occurring a mean of 23.5 days after the last treatment day. Relugolix combination therapy was generally well tolerated in premenopausal women over 84 days of treatment.

Limitations, reasons for caution: Compliance regarding tablet intake could be lower in clinical practice than in the setting of a clinical trial, therefore these results may differ from those in the clinical setting. However, the robustness of the ovarian suppression limits the risk of escape ovulations due to missed medication intake.

Wider implications of the findings: Since relugolix combination therapy completely inhibited ovulation, the use of non-hormonal contraception during treatment may not be required for prevention of pregnancy. The rapid and predictable return of ovulation after treatment discontinuation is advantageous for patients who wish to conceive after completion of treatment.

Trial registration number: EudraCT 2018-004130-15

P-288 Interventions for women with thin endometrium undergoing assisted reproductive treatment (ART)- A systematic review and meta-analysis.

L. Polanski[1], M. Baumgarten[2], K. Jayaprakasan[3]

[1]Assisted Conception Unit, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom ;
[2]Addenbrooke's Hospital, Obstetrics and Gynaecology, Cambridge, United Kingdom ;
[3]Royal Derby Hospital, Derby Fertility Unit, Derby, United Kingdom

Study question: What interventions, if any, have been shown to improve ART outcomes in women with thin endometrium defined as <7mm despite routine treatments?

Summary answer: A number of interventions have been identified. Study quality was rated as poor and live birth rates were reported by only 6 studies.

What is known already: Normal endometrial receptivity is essential for pregnancy establishment and normal development. Endometrial thickness <7 mm is associated with a reduced chance of pregnancy in ART, an increased risk of miscarriage and adverse obstetric outcomes. The aetiology of thin endometrium remains uncertain, however damage of the endometrial basal layer is the likely underlying cause. Prevalence of thin endometrium is reported in 2.4%- 8.5% of women undergoing ART, fresh or frozen cycles. Many interventions have been proposed to improve endometrial thickness and reproductive outcomes in these women.

Study design, size, duration: Design- systematic review and Meta-analysis
We identified 31 studies, comprising 2421 women, that compared treatments for thin endometrium in fresh or frozen embryo transfer cycles. Randomized controlled trials (RCTs), case controlled studies and cohort studies with a control population were included. Medline, Embase and CENTRAL databases have been systematically searched until 08.11.2019 using terms (thin OR atrophic AND endometri*) AND (assisted conception OR reproduction OR in vitro fertili*(IVF) OR intracytoplasmic sperm injection(ICSI) OR frozen embryo).

Participants/materials, setting, methods: Infertile women undergoing fresh or frozen autologous embryo transfer where endometrial thickness as defined by the authors was thin (<8mm) were included in the analysis. Control populations included women with no intervention or previous cycle data (for endometrial thickness). The main outcome was live birth, secondary outcomes included endometrial thickness, implantation rate, clinical pregnancy rate, miscarriage rate, complications of treatment and obstetric/perinatal outcomes. Mantel-Haenszel odds ration with random effects was used as analysis method.

Main results and the role of chance: A total of 350 items were identified, after deduplication 266 titles were screened. 47 papers were included for detailed analysis with 31 studies included in the meta-analysis. Identified treatments included infusion of granulocyte colony stimulating factor (G-CSF); infusion of platelet rich plasma (PRP); long term treatment with pentoxyfilline and tocopherol; pelvic flood bionic stimulation, growth hormone injections, high dose estrogen therapy, use of prostaglandins, addition of gonadotrophin releasing hormone agonists (GNRH-a) as luteal phase support.
Live birth rate was reported by 6 studies with addition of GnRH-a to luteal phase showing an odds ratio (OR) of 8.80 [95% Confidence Intervals(CI) 2.44-31.73, P=0.0009, one study). Most significant increase in endometrial thickness was noted with long treatment with pentoxifylline and tocopherol (mean difference 2.40, 95% CI 2.15-2.65, P<0.00001, one study). Endometrial perfusion with PRP demonstrated highest chance of implantation (OR 4.92, 95% CI 1.78-13.58, P=0.002, two studies). Addition of GnRH-a to luteal support demonstrated highest chance for clinical pregnancy (OR=3.76; 95% CI 1.51-9.36, P=0.004, one study). No complications or obstetric outcomes were reported.
Based on the risk of bias, only one study rated as good with majority rated as poor. Heterogeneity ranged from I^2=0 (low) to >80% (high).

Limitations, reasons for caution: Only 10 randomized controlled trials were identified with the largest including only 135 patients. The quality of the studies was mainly poor to moderate. Studies reporting other therapies identified by the search did not have adequate data to carry out meta-analysis.

Wider implications of the findings: Our study highlights the lack of good quality evidence for different types of treatments for women with persistently thin endometrium. Addition of GnRH-a to luteal phase support shows some promise however, larger studies are necessary to validate this finding. Other therapies require more well-designed RCTs to confirm their clinical benefit.

Trial registration number: PROSPERO registration number CRD42019154704.

P-289 The effect of endometriosis in the chromosomal status and developmental competence of cleavage-stage embryos. Differences with embryos in the blastocyst stage.

P. Hernandez[1], B. Gadea[2], V. Lozoya[3], V. Legidos[4], M. Muñoz[4]

[1]IVI Alicante, Local Research Department, Alicante, Spain ;
[2]IVI Alicante, IVF Laboratory, Alicante, Spain ;
[3]IVI Foundation, Research Support and Management UAGI, Alicante, Spain ;
[4]IVI Alicante, Gynecologist, Alicante, Spain

Study question: Is there any difference in the PGT-A results between trophectoderm and blastomere biopsies from patients with endometriosis compared to control patients?

Summary answer: Unlike PGT-A results in blastocyst, the number or euploid cleavage-stage embryos is lower in endometriosis patients but it does not affect embryo developmental arrest.

What is known already: Endometriosis affects around 30-50% of infertile women undergoing fertility treatment and negatively impacts on their chances of successful IVF outcomes. Among the underlying mechanism responsible for the poor outcomes, oocyte meiotic spindle alterations and chromosomal instabilities, subsequently resulting in embryo aneuploidies, have been suggested. The only data available for human embryo chromosomal analysis suggested that endometriosis does not impact on aneuploidy rates but only embryos reaching the blastocyst stage were included in the analysis. Therefore, aneuploidies leading to embryonic developmental arrest before the blastocyst stage may not have been detected.

Study design, size, duration: Multicenter retrospective cohort study to evaluate the chromosomal status of both cleavage-stage and blastocyst embryos from patients with endometriosis (n=507) compared with disease-free control women (n= 7969). Women aged 18-42 years old undergoing IVF with preimplantation genetic testing for aneuploidies (PGT-A) in IVIRMA Clinics between 2012 and 2019 were included. Both, developmental competence and PGT-A results were also examined in the cleavage-stage biopsied embryos to ascertain the effect of endometriosis on aneuploidy-related embryonic arrest.

Participants/materials, setting, methods: Presence of endometriosis was evidenced at the time of abdominal surgery or after pelvic ultrasound or NMR.

Severe male factors and patients with chromosomopathy in previous embryos or pregnancies were excluded. Both D3 and D5-7 biopsies were analyzed using comparative genomic hybridization (CGH) arrays or next generation sequencing (NGS). For the statistical analysis, chi-square test for categorical variables or Student's t-test for quantitative data were applied to compare baseline characteristics between groups.

Main results and the role of chance: Two thousand seventy-two embryos from patients with endometriosis were analyzed and 32008 were included in the control group. Among them, 9049 were cleavage-stage embryos (943 and 8106 embryos in endometriosis and control group, respectively) and 25031 were blastocyst (1129 and 23902 blastocysts from patients with endometriosis and control women). The mean number of euploid embryos from patients with endometriosis (1.04; CI: 0.93-1.15) tends to be lower than in the control group (1.17; CI: 1.14-1.2). We only found significant differences in the number of euploid embryos when results from D3-biopsies were considered (0.94 vs. 1.18; p=0.0084 in endometriosis vs. control group). Although no significant differences were shown, a similar trend was observed when ploidy rates from D3 embryos were analyzed (euploid rates: 0.18 vs. 0.21 in endometriosis vs. control group; p= 0.08). In D3-biopsied embryos, we also analyzed the ability of blastocyst formation and its relationship with their ploidy status. Similar frequencies of embryo arrest before reaching the blastocyst stage were found in both groups (20.13 % vs. 22.66 % in endometriosis and control group; p=0.09). Focusing on the PGT-A results, data showed no differences in the ploidy status when arrested embryos from both patients' groups were compared (OR: 1.12; (0.51-2.02)).

Limitations, reasons for caution: Although the study groups were as homogeneous as possible, results should be taken into caution due to the retrospective nature of the study. Multivariate regression models to avoid confounding variables are still pending. Differences in ploidies according to the severity of the disease have not been evaluated.

Wider implications of the findings: Further research is needed to firmly convey no association between endometriosis and embryo aneuploidy rates. Prospective and well-designed studies stratifying PGT-A of women with different stages of endometriosis are required. Moreover, association between aneuploidy and embryo developmental arrest should be considered in the analysis.

Trial registration number: Not Applicable

P-290 Effects of the levonorgestrel intrauterine system on the endometrium after long-term exposure to mifepristone: secondary outcomes of a randomized controlled trial

K. Papaikonomou[1], F. Caroline[1], A.R. Williams[2], K. Gemzell Danielsson[1]

[1]Karolinska Institutet and Karolinska University Hospital, Department of Women's and Children's Health- Division of Obstetrics and Gynecology, Stockholm, Sweden ; [2]The Royal Infirmary of Edinburgh- The University of Edinburgh, Division of Pathology, Edinburgh, United Kingdom

Study question: Is direct placement of a levonorgestrel intrauterine system (LNG-IUS), after long-term treatment with the progesterone receptor modulator (PRM) mifepristone, a safe alternative regarding the endometrium?

Summary answer: LNG-IUS placed immediately following long-term treatment with a PRM without any prior endometrial shedding may represent a safe treatment regimen for the endometrium.

What is known already: Clinical trials on long-term administration of mifepristone have demonstrated its potential use for contraception, medical treatment of uterine fibroids, endometriosis, improvement of bleeding patterns in women using progesterone only contraceptives and optimization of in vitro fertilization treatment. Ulipristal acetate, another PRM, is licensed for treatment of uterine fibroids and more PRMs are under development. However, continuous administration of PRMs is associated with a risk of developing a characteristic endometrial morphology described as progesterone receptor modulator associated endometrial changes (PAEC). Although accumulating evidence implies that this histological entity is benign, its long-term safety profile remains uncertain.

Study design, size, duration: We report the secondary outcome from a double-blinded randomized controlled trial conducted from November 2009 to January 2015. Eligible women were randomized to 50mg mifepristone (n=29) or the comparator (vitamin B) (n=29) for two months, followed by insertion of the LNG-IUS 52mg. Endometrial biopsies were obtained at baseline and three months after placement of the LNG-IUS, with the device in situ. Paired samples were histologically assessed by an expert pathologist blinded to the treatment.

Participants/materials, setting, methods: The study was conducted in a single center in Sweden. Eligible were healthy women opting for a LNG-IUS, aged 18-43, with normal menstrual cycles. The paired endometrial samples were stained with hematoxylin and eosin and assessment involved classifying biopsies according to (1) primary diagnosis (benign, hyperplastic or malignant), (2) whether appearances represented a physiological state or not and if physiological, record the stage of the menstrual cycle and (3) the presence or absence of PAEC.

Main results and the role of chance: This is the first study assessing endometrial safety and presence of PAEC after long-term treatment with a PRM with subsequent placement of an LNG-IUS with no prior endometrial shedding. There were no differences in baseline characteristics between the groups. Nine paired biopsies in the mifepristone group and eight paired biopsies in the comparator group were included in the histological analysis after study drop-outs, loss of samples and failure in endometrial retrieval. All endometrial biopsies at baseline revealed a normal histological appearance. Endometrial biopsies after three months with the LNG-IUS in situ showed no PAEC in either group and as expected, they were consistent with progestin effects using the LNG-IUS. While PAEC are now well described, considered benign, and seem reversible after cessation of treatment, the mechanism of their development and their long-term significance remain unknown. This constitutes the reason for the clinical recommendation of treatment cessation after three months of continuous PRM use for medical treatment of uterine fibroids and endometrial shedding after PRM treatment to ensure that PAEC disappears before commencing any long-acting contraceptive method. This could though constitute a draw-back in women with uterine fibroids or endometriosis. Additionally, treatment gaps in fertile women can result in unplanned pregnancies.

Limitations, reasons for caution: Since this is a pilot study and no prior similar studies have been conducted, no specific power calculation was performed for the secondary outcome reported. Even though the sample size was small, these data still provide us with unique findings concerning the safety of endometrial morphology following this treatment regimen.

Wider implications of the findings: Our results suggest that it is safe to proceed with a long-acting progestin such as the LNG-IUS immediately after long-term PRM treatment for uterine fibroids or endometriosis. However, the endometrial safety with this or similar treatment regimens needs to be further explored in larger data settings.

Trial registration number: ClinicalTrials.gov NCT01931657

P-291 The impact of chronic endometritis on endometrial receptivity array and pregnancy rates

K. Kuroda[1], T. Horikawa[1], A. Moriyama[1], K. Nakao[1], H. Juen[1], S. Takamizawa[1], Y. Ojiro[1], K. Nakagawa[1], R. Sugiyama[1]

[1]Sugiyama Clinic Shinjuku, Division of Reproductive Medicine and Implantation Research, Tokyo, Japan

Study question: To identify the effects of chronic endometritis (CE) on endometrial receptivity array (ERA) outcomes.

Summary answer: Most patients with CE have 'non-receptive' endometrium in the ERA test.

What is known already: ERA is a novel tool for the objective identification of the personal window of implantation (WOI). CE is a chronic localized inflammatory condition of the endometrium with the CD138-positive plasma cells, yet essentially asymptomatic and undetectable by common infertility testing. Recovery from CE can lead to the improvement of pregnancy outcomes in IVF treatment. CE is associated with decreased expression of decidual markers. Therefore, CE may have adverse effects on endometrium decidualization and formation of an optimal WOI; however, the clinical impact of CE on the ERA test has not been established.

Study design, size, duration: A retrospective cross-sectional study was performed between August 2018 and October 2019 on 66 infertile women who underwent histological examinations and endometrial sampling for ERA testing. The study protocol was approved by the Ethics Committee of Sugiyama Clinic.

Participants/materials, setting, methods: Of 66 women, we recruited 56 who underwent ERA testing and immunohistochemistry (IHC) of CD138 within 3 months. We divided the subjects into 3 groups as follows: women without CE

(non-CE group, n=26), women with untreated CE at the time of ERA (CE group, n=12), and women successfully treated for CE before ERA (cured-CE group, n=18). CE diagnosis was defined as ≥5 CD138-positive cells per 10 nonoverlapping random stromal areas visualized at 400 ×magnification.

Main results and the role of chance: In the non-CE, CE, and cured-CE groups, the number of CD138-positive plasma cells per 10 random stromal areas within 3 months before or after ERA testing were 0.5 ± 0.9, 29.9 ± 31.3, and 0.9 ± 1.0, respectively. The difference in number of CD 138-positive cells between each group was found to be statistically significant ($p < 0.001$). Regarding ERA results, the rate of 'receptive' endometrium in the non-CE and cured-CE groups were 57.7% (15/26) and 55.6% (10/18), respectively; however, in the CE group, the 'receptive' endometrium rate was 8.3% (1/12), which was significantly lower than the other 2 groups ($p < 0.001$). In the CE group, 'non-receptive' endometrium was observed in most patients, including 'late or post-receptive' in 58.4% of patients, on 5 days after the initiation of progesterone administration. When CE was recognized, it was treated with antibiotic therapy prior to ET in all patients. The clinical pregnancy rates in the first ET at the time designated by ERA results in non-CE, CE, and cured-CE groups were 66.7% (14/21), 16.7% (2/12), and 50.0% (8/16), respectively ($p = 0.022$). Despite completion of CE treatment, poor pregnancy rates were observed in the CE group.

Limitations, reasons for caution: In limitations, a retrospective study and the small sample size are the main sources of bias.

Wider implications of the findings: WOI may be negatively affected by CE. And the patients may achieve optimal WOI after proper CE treatment. Therefore, screening and appropriate treatment for CE should be considered prior to ERA testing. Moreover, ERA outcomes in patients with CE may be unreliable.

Trial registration number: not applicable

P-292 Design and evaluation of a novel nanodrug delivery system for reducing the side effects of clomiphene citrate on the endometrium

M. Ajdary[1], R. Aflatoonian[2], F. Keyhanfar[3], F. Amjadi[4], Z. Zandieh , zandie.dvm@gmail.com[4], M. Ashrafi , ashrafi.m@iums.ac.ir[5], M. Mehdizadeh , mehdizadeh.m@iums.ac.ir[6]

[1]Cellular and Molecular Research Center- Iran University of Medical Sciences- Tehran- Iran, Cellular and Molecular Research Center- Iran University of Medical Sciences, Tehran, Iran ;
[2]2.Department of Endocrinology and Female Infertility- Reproductive Biomedicine Research Center- Royan Institute for Reproductive Biomedicine- Tehran- Iran, 2. Department of Endocrinology and Female Infertility- Reproductive Biomedicine Research Center-, ;
[3]Pharmacology Department- Tehran University of Medical Sciences- Hemmat Pardis- Tehran- Iran, Pharmacology Department- Tehran University of Medical Sciences- Hemmat Pardis- Tehran- Iran, tehran, Iran ;
[4]Department of Anatomical Science- Iran University of Medical Sciences- Tehran- Iran, Department of Anatomical Science- Iran University of Medical Sciences- Tehran- Iran, tehran, Iran ;
[5]Obstetrics and Gynecology Department- School of Medicine- Iran University of Medical Science- Tehran- Iran, Obstetrics and Gynecology Department- School of Medicine- Iran University of Medical Science- Tehran- Iran, tehran, Iran ;
[6]Cellular and Molecular Research Center- Faculty of Advanced Technologies in Medicine- Department of Anatomy- Iran University of Medical Sciences- Tehran- Iran, Cellular and Molecular Research Center- Faculty of Advanced Technologies in Medicine- Depart,

Study question: Is there a change in the expression of genes involved in implantation in cells treated with clomiphene citrate compared to clomiphene citrate loaded in PBF?

Summary answer: A significant difference was found between genes involved in the implantation of cells treated with clomiphene citrate compared to clomiphene citrate loaded in PBF.

What is known already: Growing evidence suggests that a disorder of endometrial receptivity may contribute to the adverse reproductive outcomes in the stimulation of ovulation by clomiphene citrate (PCOs). And by developing a slow-release property in lipophilic drugs, Phospholipids can prevent the damage of lipophilic drugs to non-target tissues.

Study design, size, duration: This study was a basic genomic analysis of human endometrial biopsies taken from eight healthy fertile women in the secretory phase.

Participants/materials, setting, methods: PBF containing a mixture of phosal 50PG, glycerol and deionized water was prepared using a probe sonicator. In vitro model, human endometrial cell were into four groups. The control group was incubated only with DMEM medium containing antibiotics and 10% FBS and the experimental groups *co-cultured* with sperm, *co-cultured* with sperm and clomiphene citrate, *co-cultured* with sperm and nano-clomiphene citrate were incubated for 24 h and the next day, cells were harvested for q-PCR.

Main results and the role of chance: The optimized PBF contained Phosal 50PG/glycerol in a 2:8 ratios (w/w) and mean particle size of Nano drug used were 67±9.0 nm, the release of CC from Nano emulsion was slightly faster in the first 24 hours; during this period, 29% of CC was released. After 120 hours 76% of CC was released. The mean mRNA level of MUC1 gene was significantly increased in CC and CC / PBF group compared to control ($P < 0.001$). The mean mRNA level of PGR gene was significantly increased in the sperm-treated group ($P < 0.05$) and in the sperm and PBF group compared to CC ($P < 0.001$). The mean mRNA level of VEGF gene in sperm and PBF group was significantly increased ($P < 0.05$) compared to control and CC / PBF was significantly increased ($P < 0.001$) compared to CC and control.

Limitations, reasons for caution: The main limitation of this study is a low number of human endometrial samples

Wider implications of the findings: Formulation sustained release of clomiphene citrate increased its targeting efficiency and improved the impact of the CC on the serum levels of estradiol and expression of genes involve implantation. A new Phosal-Based Formulation (PBF) was introduced to decrease side effect of clomiphene citrate on endometrium.

Trial registration number: 6736

P-293 Use of a Vaginal Probiotic Suppository and Antibiotics in the Treatment of endometrial microbiota.

D. Kadogami[1], Y. Nakaoka[1], Y. Morimoto[2]

[1]IVF Namba Clinic, medical office, Osaka, Japan ;
[2]HORAC Grandfront Osaka Clinic, medical office, Osaka, Japan

Study question: What is an effective treatment for endometrial microbiota?

Summary answer: The combination use of vaginal probiotic suppository and antibiotics was effective for the treatment of endometrial microbiota.

What is known already: The development of next-generation sequencing (NGS) technologies has made it possible to quantify subdivided bacteria using the variable region of the 16S rRNA gene, enabling a more comprehensive assessment of the microbiota. Through this innovation, various studies have focused on the relationships between the female genital microbiota and pregnancy. Some authors evaluated the endometrial microbiota of IVF patients using NGS and showed that the group with 90% or higher *Lactobacillus(LD)* had significantly higher implantation rates and pregnancy rates than those with less than 90% *Lactobacillus(NLD)* . Therefore, new strategies are needed to improve pregnancy rates in patients with NLD.

Study design, size, duration: Randomized controlled trial in our clinic, between December 2018 to October 2019.
Evaluation of the endometrial microbiota was performed in 224 patients with RIF. NLD cases were randomly assigned to treatment groups that combine Lactoferrin(oral prebiotic), Lebenin(oral probiotic), inVag(vaginal probiotic suppository) and Metronidazole in various ways. Re-analysis was performed after treatments and results were presented as healing rates. The healing rate was defined as the rate of cured cases among treated cases.

Participants/materials, setting, methods: The treatment protocol was determined as follows. Protocol A was Lactoferrin(300mg/day for 30days) and Lebenin(3g/day for 30days), B was Metronidazole(vaginal suppository 250mg, only once and oral 750mg/day for 7days), C combined A and B, D was Lactoferrin(600mg/day for 30days) and Lebenin(3g/day for 30days) and Metronidazole (vaginal suppository 250mg and oral 750mg/day for 7days), E was inVag(1capsule/day for 7days), F was inVag (1capsule/day for 7days) and Metronidazole(vaginal suppository 250mg and oral 750mg/day for 7days).

Main results and the role of chance: Evaluation of the endometrial microbiota was performed in 224 patients with RIF. LD was found in 114 patients (50.9%), whereas NLD was found in 110 patients (49.1%). There were no significant differences in patient backgrounds between groups. In NLD cases, *Gardnerella* was

the most abundant bacteria, and *Atopobium*, *Streptococcus*, and *Prevotella* were detected abundantly, after excluding *Lactobacillus* and *Bifidobacterium*.
About protocol A, B and C, the healing rate was only 30%, and there were no significant differences in healing rate between treatment groups ($P = 0.91$ and $P = 0.60$, respectively). Especially in A, there were no cured cases among patients those *Lactobacillus* proportion was less than 70% before treatment. These results suggested that oral prebiotics and probiotics alone were not sufficient to improve the endometrial microbiota and that antibiotics were necessary. Between C and D, there were no significant difference in healing rate (33.3% versus 32.5%, respectively, $P = 1.00$).

However, higher healing rate was reported in E compared with D(32.5% versus 43.6%, respectively, $P = 0.39$). Further, F had a significantly higher healing rate compared with D(32.5% versus 72.7%, respectively, $P = 0.02$).

Throughout this study, no side effects that were likely related to the medication were observed.

Limitations, reasons for caution: Assessment of the endometrial microbiota requires transvaginal collection of endometrial fluid using a catheter tube, which may cause contamination. Moreover, although NGS is useful for quantifying bacteria, it only measures the bacterial 16S rRNA gene and does not guarantee bacterial viability.

Wider implications of the findings: The endometrial microbiota was evaluated in patients with RIF, and 49.1% of cases had less than 90% *Lactobacillus* . In such cases, probiotics combined with vaginal probiotic suppository and antibiotics proved to be highly effective. In our future studies, we will examine pregnancy rates after treatment of the endometrial microbiota.

Trial registration number: UMIN-CTR 000038582

P-294 Surgical management of deep endometriosis and in vitro fertilization outcomes: A systematic review and meta-analysis

B. Woolnough[1], C. Qianqian Wu[1], T. Vause[1], H. Shenassa[1]

[1]University of Ottawa, Obstetrics and Gynecology, Ottawa, Canada

Study question: In women with deep endometriosis (DE), does surgery followed by in vitro fertilization (IVF) improve fertility outcomes compared to first-line IVF?

Summary answer: Considering limited evidence, surgical management of DE before IVF is associated with improved pregnancy rates but similar live birth rates when compared with first-line IVF.

What is known already: Surgical management of DE in infertile women prior to in vitro fertilization (IVF) is controversial. Surgeries are often invasive and may cause significant complications. Recent evidence suggests that surgery may improve IVF outcomes, however studies have been small and underpowered.

Study design, size, duration: We conducted a systematic review and meta-analysis, which identified studies including a total of 538 women. We searched MEDLINE, EMBASE and the Cochrane Library (inception to October 2019) for studies comparing fertility outcomes in women with DE with and without surgical treatment prior to IVF.

Participants/materials, setting, methods: The study population included all identified women with deep endometriosis who received treatment of either surgery followed by IVF or IVF alone. Study selection, data extraction and quality assessment were conducted independently by 2 reviewers. Meta-analysis was conducted using a random effects model.

Main results and the role of chance: Four studies meeting the inclusion criteria were identified, one prospective and three retrospective cohort studies. Three of these were able to be included in the quantitative meta-analysis. Clinical pregnancy rate was significantly higher in the surgery group (risk ratio [RR] = 1.41; 95% CI = 1.03 to 1.95; P = 0.03; $I^2 = 25\%$). Live birth rates were comparable between the groups (RR = 1.60; 95% CI = 0.97 to 2.62; P = 0.06; $I^2 = 48\%$). The total number of oocytes retrieved, gonadotropin dose used, and miscarriage rates were also similar between the two groups.

Limitations, reasons for caution: The retrospective nature of the included studies permits introduction of bias, limiting the conclusions that can be made.

Wider implications of the findings: The potential benefit of surgery must be weighed against risk, including potential reduction in ovarian reserve. While this study suggests similar outcomes, well-designed and appropriately-powered randomized controlled trials are needed to determine if there is any clinical advantage to surgical management of DE prior to IVF.

Trial registration number: not applicable

P-295 Sonographic Quantification of Myometrial Conditions in Adenomyosis Patients with Computer Assisted Image Analysis-A Pilot Study

S.Y. Chou[1], L. Buo Jia[1], C. Yi-En[1], C. Cindy[1], C. Ching Hui[1], C. Chi-Huang[1]

[1]Taipei Medical University Hospital, Reproductive medicine and science, Taipei, Taiwan R.O.C.

Study question: We examined the diagnostic feasibility of using artificial neural network (ANN)-analyzed sonographic gray-scale histograms to assess myometrial conditions based on serum cancer antigen 125 (Ca-125) levels in patients with adenomyosis.

Summary answer: By analyzing the gray scale histograms of the myometrium on ultrasonographic images, this quantitative approach may help in the initial diagnosis of adenomyosis.

What is known already: The only definitive method for the diagnosis of adenomyosis is to perform a hysterectomy combined with histological examination. However, there is no universal agreement on the exact histological criteria, and the diagnostic methodcannot be made prior to invasive surgery.

The accuracy of using only a serum Ca-125 assay to diagnose adenomyosis remains limited, Ca-125 can help in the initial screenings of women with possible adenomyosis
Ultrasonography technology is a quick, safe, easy to use, and inexpensive modality that makes it possible to recognize adenomyosis without surgery. However, there are no standard diagnostic imaging criteria for adenomyosis.

Study design, size, duration: This was a retrospective observational study of 26 female patients attending Taipei Medical University Hospital, Taiwan for infertility between September 1, 2018 and March 31, 2019.The research protocol of this study was approved by the Institutional Review Board of Taipei Medical University Hospital (IRB number: N201711084).

Participants/materials, setting, methods: A total 54 ultrasonographic images of the myometrium were divided into three groups based on serum Ca-125 levels:15 images from patients with Ca-125 values <35 U/ml, 19 images from patients with Ca-125 values between 35 and 100 U/ml, and 20 images from patients with Ca-125 values >100 U/ml. Each patient with the same machine and settings, and sonographic gray-scale histograms of the endometrium were analyzed with ImageJ image processing software using the ANN model.

Main results and the role of chance: ANN classification was used to characterize the sonographic data. Although the median signal intensity score of the Ca-125>100 group (2333811) was higher than that of the Ca-125<35 group (1652460) and the 35≤Ca-125≤100 group (1915953), Kruska-Wallis statistical analyses indicated no significant difference among groups (p=0.55).

The Youden index is a measure for evaluating the effectiveness of a biomarker. The cut-points of signal intensity scores among the three groups according to the Youden index and their sensitivity and specificity in predicting adenomyosis probability are shown. For the comparison between Ca-125<35 and 35≤Ca-125≤100, the sensitivity and specificity were 36.8% and 86.7%, respectively; for the comparison between Ca-125<35 and Ca-125>100, the sensitivity and specificity were 40% and 93.3%, respectively; for the comparison between 35≤Ca-125≤100 and Ca-125>100, the sensitivity and specificity were 35% and 84.2%, respectively.

This quantitative approach had a high specificity, which might be useful in the early diagnosis of adenomyosis.

Limitations, reasons for caution: This study was highly operator-dependent and did not detract from the observational experience required in ultrasonographic examinations.

The diagnosis was made by ultrasonographic examination, with no histopathological confirmation, which may limit the accuracy of our findings.
The small sample size and irregular distribution of baseline characteristics may have influenced the results.

Wider implications of the findings: In our study, not only the size and volume of the uterus but also its contents and textures were accessed quantitatively by analyzing the gray-scale histogram of the myometrium. Our approach is of great assistance in the initial screening of women with suspected adenomyosis.

Trial registration number: N201711084

P-296 3D-Saline Contrast Sonohysterography in detecting uterine anomalies and pathologies among sub-fertile women and women with AUB

D. Al-Jaroudi[1], F. Khan[1], M. Al-Tannir[2], I. Al Fayyad[2]

[1]King Fahad Medical City, Women's Specialized Hospital, Riyadh, Saudi Arabia ;
[2]King Fahad Medical City, Research center, Riyadh, Saudi Arabia

Study question: Does 3D-SCSH detect both congenital uterine anomalies and uterine pathologies in sub-fertile and AUB patients?
Summary answer: 3D-SCSH detects both congenital uterine anomalies and uterine pathologies in sub-fertile and AUB patients and is a tool to be used when suspecting uterine pathology.
What is known already: Uterine cavity anomalies could be a contributing cause of subfertility in women, and abnormal uterine findings are reported in as many as 50% of women with recurrent implantation failure.
Study design, size, duration: A prospective cohort study was conducted over three years. Total number of 3D-TVS performed was 2,222. 1477 women were from the sub-fertile group and 745 patients from AUB group.
Participants/materials, setting, methods: A prospective cohort study was conducted over three years. The women were recruited from the outpatient department and Reproductive Endocrinology and Infertility Medicine Department. All women of reproductive age group, either with abnormal uterine bleeding (AUB) or sub-fertile underwent 3D-TVS. If initial 3D-TVS detected any uterine abnormality, then the patient was booked for 3D-SCSH. Uterine anomalies were recorded according to the new ESHRE/ESGE classification of uterine anomalies and compared in the two groups.
Main results and the role of chance: Results: Total number of 3D-TVS performed was 2,222. 1477 women were from the sub-fertile group and 745 patients from AUB group. Total of 330 women required 3D-SCSH, of whom 215 (65%) were sub-fertile women, and 115 (35%) were AUB. Uterine anomalies were found in 15 (7%) in the sub-fertile group compared to 2 (1.7%) In the AUB group. The partial septate uterus was the most common finding among the sub-fertile group 7 (3.3%) versus 2 (1.7%) with dysmorphic uterus in the AUB group. Uterine polyps were the commonest uterine pathology in both groups.
Conclusion: 3D-SCSH may detect congenital uterine anomalies in sub-fertile and AUB patients. This diagnostic accuracy may support the adoption of 3D-SCSH as a routine investigation in the evaluation of sub-fertile women prior to IVF.
Limitations, reasons for caution: NA
Wider implications of the findings: The prevalence of uterine anomalies among sub-fertile women was found to be higher in comparison to women with AUB. 3D-TVS and 3D-SCSH are accurate, and safe in determining the prevalence of congenital uterine anomalies in sub-fertile women.
Trial registration number: NA

P-297 Introducing intrauterine antibiotic infusion as a means towards eradicating chronic endometritis and restoring reproductive dynamics: A randomized pilot study

K. Sfakianoudis[1], M. Simopoulou[2], E. Maziotis[2], A. Rapani[2], P. Tzonis[1], N. Nitsos[1], P. Giannelou[2], S. Grigoriadis[2], P. Tsioulou[2], M. Koutsilieris[2], K. Pantos[1]

[1]Genesis, Assisted Conception Unit, Chalandri- Athens, Greece ;
[2]National and Kapodistrian University of Athens, Physiology, Athens, Greece

Study question: Does intrauterine antibiotic infusion in combination with per os administration in patients with Chronic Endometritis (CE) enhance treatment and fertility status?
Summary answer: The combination of oral administration and intrauterine infusion of antibiotics provided enhanced treatment results when compared to oral antibiotic administration for CE patients.
What is known already: An infectious endometrial environment caused by certain pathologies could compromise pregnancy rates for patients pursuing fertility treatment. In the case of persistent endometrial inflammation, a diagnosis of CE may be established. Oral antibiotic administration constitutes the gold standard approach for treating CE cases and has been confirmed to contribute to a clearance rate, albeit without adequate treatment rate.
Study design, size, duration: Women included in the present study were referred for CE investigation following either repeated implantation failure (RIF) (N=40) or recurrent miscarriage (RM) (N=40) originating from natural conception, without any other infertility aetiology. Following diagnosis of CE, patients were randomized into two groups, one receiving oral treatment and the other receiving a combination of oral antibiotics and intrauterine infusion, stratified by the referral diagnosis. Enrolment of patients was performed from March 2017 till February 2019.
Participants/materials, setting, methods: Diagnosis of CE and treatment evaluation were performed by employing hysteroscopic investigation, endometrial biopsy, along with histological analysis and microbiological culture. Patients following successful treatment proceeded with pursuing a pregnancy via natural conception or IVF, according to the original referral diagnosis. Patients presenting with RIF proceeded with a single IVF cycle. Patients presenting with RM were invited to conceive naturally over the course of six months. The study was conducted in a single IVF center.
Main results and the role of chance: The combined oral and intrauterine administration of antibiotics provided statistically significant improved results regarding efficiency of treatment when compared to oral antibiotic administration only (34/40vs25/40; RR:1.36; 95%CI:1.04-1.79; p=0.04). It should be mentioned that the number of treatment days was also statistically significantly increased (29.93vs15.00; p<0.001) in the combination group. Regarding the IVF arm no statistically significant difference was indicated between the combination group (13/18) and the oral administration (9/13) regarding neither clinical pregnancy (CP) (13/18vs9/13; RR:1.04; 95%CI:0.66-1.66; p=0.85) nor live-birth (LB) (13/18vs8/13; RR:1.17; 95%CI:0.70-1.96; p=0.70). Intention-to-treat (ITT) analysis did not alter the results regarding neither CP (RR:1.18; 95%CI:0.71-1.97; p=0.75) nor LB (RR:1.63; 95%CI:0.87-3.04; p=0.20). Regarding the natural conception arm no statistically significant difference was presented between the combination group (11/17) and the oral administration group (7/12) regarding neither CP (11/17vs7/12; RR:1.12; 95%CI:0.66-1.66; p=0.85) nor LB (10/17vs7/12; RR:1.00; 95%CI:0.54-1.87; p=0.98). ITT did not alter the results regarding neither CP (RR:1.49; 95%CI: 0.80-2.78; p=0.34) nor LB (RR:1.35; 95%CI 0.73-2.50; p=0.52). Pooling of data presented a marginally statistically significant improvement in the combination group compared to oral administration regarding both CP (25/40vs16/40; RR:1.56; 95%CI:1.00-2.45; p=0.05), and LB (24/40vs15/40; RR:1.58; 95%CI:1.00-2.05;p=0.05).
Limitations, reasons for caution: The small sample size along with the fact that the study was carried out in a single center are limitations for the study. Moreover, since CE is regarded as a Sexually Transmitted Disease the lack of follow-up regarding the male partner stands as a reason for caution.
Wider implications of the findings: Results indicate that intrauterine antibiotic infusion may hold the key to a long-term effective treatment, extending to establishing a favorable basis for achieving pregnancy for patients diagnosed with CE and subsequent infertility. This study establishes preliminary grounds for conducting research focused on a pharmaceutically developed drug appropriate for endometrial administration.
Trial registration number: Not applicable

P-298 Expression of claudin 3 and caludin 4 in deep endometriosis

M. Mendonça Carneiro[1],[2,3], P. Vidigal[4], L.P. Costa[3], C. Moreira[1], T. Moreira[1], V. Campos[1], P. Gouvea[3], T. Pardini[1], A. Silva Filho[1], I. Ávila[3]

[1]Universidade Federal de Minas Gerais, Obstetrics and Gynecology, Belo Horizonte, Brazil ;
[2]Rede Mater Dei de Saúde, Center for Human Reproduction, Belo Horizonte, Brazil ;
[3]Biocor Instituto, Endometriosis Multidisciplinary team, Belo Horizonte, Brazil ;
[4]Universidade Federal de Minas Gerais, Pathology, Belo Horizonte, Brazil

Study question: To evaluate the association between immunoexpression of claudin 3(CLND-3) and 4 (CLDN-4) with clinical, surgical and biochemical data in women with deep endometriosis (DE).
Summary answer: DE expresses both CLDN-3 and CLDN-4. Expression was weak and not influenced by clinical parameters. CLDN-3 expression was significantly higher and related to disease extension.
What is known already: DE is characterized by lesions which extend more than 5 mm underneath the peritoneum and is responsible for painful symptoms. Numerous molecular abnormalities have been related to DE pathogenesis including immunological, inflammatory, genetic and hormonal alterations. Claudins are

the main junctional proteins between epithelial cells and may be may play a role in tissue remodeling and invasion. CLND-3 and CLDN-4 have been studied in endometrial hyperplasia and cancer with growing evidence showing a possible contribution in their pathogenesis and thus prompted studies to assess their possible role in endometriosis. CLDN3 and 4 have not been studied in DE so far.
Study design, size, duration: women undergoing surgical treatment of DE at a tertiary center (Biocor) with confirmed histological diagnosis from August/2011 to Decemer/2016 comprised the study group whereas the control group included women undergoing surgery for other benign gynecological conditions (pelvic organ prolapse or uterine fibroids) who gave their consent to participate. The study protocol was approved by the local ethics committee.
Participants/materials, setting, methods: Samples were otained during surgery in both groups and prepared for IHC using antibodies for both CLDN-3 and CLDN-4. IHC expression was considered present (+) or absent (-) and intensity classified into three categories: weak (+), moderate (++) and strong (+++ and ++++). IHC was performed with the Streptavidin-Biotin protocol using antibodies against CLDN3 and CLDN4. The data was analysed using the Z test for proportions, Fisher's exact chi-square test and an ordered logistic regression.
Main results and the role of chance: This case-control study involved immunohistochemical evaluation (IHC) of 48 samples from 36 patients undergoing surgical endometriosis treatment at a tertiary center by a multidiscplinary endometrisis team.Thirty-five (73%) subjects had DE diagnosis. CLDN-3 expression was positive in 60% of DE and 80% of CLDN-4 samples. Expression was higher in the intestines (CLDN-3: p 0.016; CLDN-4: p 0.000). In other sites a weak intensity expression predominated, both from CLDN-3 (75.4%) and CLDN-4 (73.8%). Expressions of CLDN-3 and CLDN-4 were not associated with preoperative hormone use, body mass index (BMI), vitamin D, and serum 125 (CA125) antigen. CLDN-3 showed more positivity in the more advanced stages of endometriosis (moderate and severe). CLDN-3 expression was significantly higher than in controls ($p < 0.001$)
Limitations, reasons for caution: Although DE lesions expressed both CLDN 3 and 4, the sample is small and comes form a single center. Claudin 3 and 4 expression was studied only by IHC.
Wider implications of the findings: CLDN-3 expression was significantly higher than in controls and showed more positivity in the more advanced stages of endometriosis (moderate and severe). The role of CLDN3 in DE remains to be established.
Trial registration number: NOT APPLICABLE

P-299 Assessing the endometrium in recurrent implantation failure (RIF) – a prospective controlled cohort study

M. Hviid[1], T. Hallager[2], G. Persson[3], L. Grupe[2], K. Birch Petersen[4], T.V. Hviid[3], N.S. Macklon[4]

[1]Zealand University Hospital, Department of Gynaecology- Obstetrics- and Fertility, Roskilde, Denmark ;
[2]Zealand University Hospital, Pathology Department, Roskilde, Denmark ;
[3]Zealand University Hospital, Department of Clinical Biochemistry, Roskilde, Denmark ;
[4]Zealand University Hospital, Department of Gynaecology- Obstetrics and Fertility, Roskilde, Denmark

Study question: What markers of endometrial dysfunction can be identified among women experiencing RIF and do they differ compared to control patients?
Summary answer: Women with RIF reveal a range of endometrial dysfunctions different in prevalence from control patients.
What is known already: The endometrial factor is recognized as a cause of RIF. However, management remains largely empirical and of questionable efficacy. There is a need to guide therapy by making an endometrial diagnosis where possible.
Study design, size, duration: Prospective controlled cohort study. Between November 2017 – September 2019, 86 women with a history of RIF (defined as failure to conceive after at least three transfers of high quality embryos), referred to a University Hospital-based 'Implantation Clinic', and 37 women with no history of RIF underwent timed endometrial diagnostic profiling in a hormone substituted cycle. Samples were collected throughout the span of a year.
Participants/materials, setting, methods: Endometrial and blood profiling was carried out in all participants in a hormone substituted cycle using estradiol and progesterone and sampled after five days of progesterone treatment. Endometrial biopsies were analysed by histology, immune cell profiling (CD56, CD16, CD163, CD138, regulatory T cells), and gene expression (ERA® test, Igenomix, Spain). The vaginal microbiome was analysed using a NGS technology (ArtPRED®, The Netherlands). Blood tests included estradiol, progesterone, prolactin, TSH, anti-phospholipid screening, and vitamin D.
Main results and the role of chance: Patients with RIF demonstrated different individual endometrial profiles, indicating that a 'one treatment fits all' approach is inappropriate. Compared with controls, women with RIF demonstrated a higher prevalence of chronic endometritis as defined by cells staining positive for CD138 by immunohistochemistry (25% vs. 3% in the control cohort, $p = 0.004$), a lower mean level of vitamin D (43 nmol/L vs. 51 nmol/L; $P = 0.04$), and a borderline lower mean mid-luteal phase progesterone (39 nmol/L vs. 44 nmol/L; $P = 0.08$). Compared with controls, women with RIF appeared to have a higher prevalence of a vaginal microbiome reported to be predictive of implantation ($P = 0.04$). While the RIF cohort was slightly older than the controls (mean age 33.8 years vs. 30.2 years $P = 0.0001$) no differences between the two groups were observed with regard to AMH, BMI, anti-phospholipid antibody prevalence, mean mid-luteal estradiol, prolactin, TSH levels, number of CD56 cells, CD16 cells, regulatory T cells or macrophages present in the mid-luteal endometrial biopsy.
Limitations, reasons for caution: The tests performed in the study were taken in a standardised hormone substituted cycle and results should be interpreted in that clinical context. The control cohort consisted of women referred for fertility treatment with male factor infertility or tubal factor, hence not representing a proven fertile population.
Wider implications of the findings: Diagnosing the endometrium in women with RIF permits targeted rather than blind empirical interventions. Relative Vitamin D deficiency, lower mid-luteal progesterone, and chronic endometritis are ready targets for treatment. Understanding the role and treatment of an unfavourable vaginal microbiome needs further investigation.
Trial registration number: REG-117-2017

P-300 Differences in the effects of metformin treatment on viability and migration of endometrial cancer cells in a normal or high glucose environment

C. Lange[1], A. Machado Weber[1], T. Strowitzki[1], A. Germeyer[1]

[1]University Women's Hospital Heidelberg, Department of Gynecological Endocrinology and Fertility Disorders, Heidelberg, Germany

Study question: Does metformin treatment have effects on cell viability and migration of endometrial cancer cells and do these effects vary in a normal or high glucose environment?
Summary answer: Metformin decreased cellular viability and migration in endometrial cancer cell lines. However, variation of glucose levels had no effect on cellular viability and migration.
What is known already: Endometrial cancer (EC) is one of the most common gynecological malignancies and can occur in an estrogen-dependent (type I, 70 – 80 %) or a more aggressive estrogen-independent form (type II). High estrogen levels, e.g. due to metabolic syndrome and associated polycystic ovary syndrome (PCOS), or hyperglycemia, e.g. in due to diabetes, increase the risk of developing EC and lead to higher mortality, especially in obese women. Metformin, an anti-hyperglycemic and insulin sensitizing drug, is used in the treatment of type II diabetes mellitus. However, anti-cancer effects have been observed in several studies, but detailed information is lacking for EC.
Study design, size, duration: In the present *in vitro* study, EC cells were cultured in normal (NG; 5.5 mmol/L, equivalent to 100 mg/dL) or high (HG; 17.0 mmol/L, equivalent to 306 mg/dL) glucose media (supplemented with 10 nmol/L ß-estradiol) and treated with metformin (0.1–20.0 mmol/L) over a period of 7 d. Cells were subsequently analyzed for changes in cell viability and migration rates. Untreated cells served as controls. Data was obtained from at least three independent experiments.
Participants/materials, setting, methods: The study was carried out with the two different EC cell lines HEC-1A (type II) and Ishikawa (type I). The analysis of cellular viability was based on mitochondrial dehydrogenase activity in the

MTT assay. The migration of the cells was analyzed by changes in scratch area in the wound healing assay.
Main results and the role of chance: A dose-dependent decrease of cellular viability was observed for HEC-1A and Ishikawa cells after metformin treatment, irrespective of glucose levels. At a concentration ≤0.1 mmol/L, viability remained ≥80% compared to untreated controls, both in a normal and high glucose environment. Viability dropped to ≤10% at concentrations >5.0 mmol/L (HEC-1A) and 10.0 mmol/L (Ishikawa) under any tested condition. IC_{50} values for Ishikawa cells were 5.10 mmol/L (NG; IC_{90}: 44.4 mmol/L) and 3.90 mmol/L (HG; IC_{90}: 33.7 mmol/L). For HEC-1A cells, an IC_{50} value of 0.75 mmol/L (NG; IC_{90}: 6.83 mmol/L) was established. The wound healing assay revealed that the ability to migrate into the scratch area decreased with increasing metformin concentrations, but independent of glucose levels. For HEC-1A control cells, 70% (NG) and 56% (HG) of the scratch area was covered with cells 24 h after wounding of the cell monolayer. After treatment with 0.5 mmol/L metformin, the wounded area was invaded by 53.4% (NG) and 45.4% (HG), respectively, and only by 17.7% (NG) and 20.8% (HG) at 5.0 mmol/L metformin. The same effect was observed with Ishikawa cells, although migration was generally lower. Metformin at 5.0 mmol/L even led to a small enlargement of the wound area by 2.6% (HG).
Limitations, reasons for caution: The results are obtained in an *in vitro* study with human cancer cell lines, and thus cannot be easily extrapolated to patients.
Wider implications of the findings: Results suggest that metformin treatment can lower the migration ability of EC cells, especially of the more aggressive type II. Therefore, metformin may also be able to prevent EC development in patients at high risk, e.g. with high estrogen levels (metabolic syndrome and PCOS), hyperglycemia (type II diabetes) or obesity.
Trial registration number: not applicable

P-301 E-cigarettes smoke impairs endometrial receptivity

A. La Marca[1], S. Sacchi[1]

[1]University of Modena and Reggio Emilia- and Clinica Eugin, Mother-Infant Department, Modena, Italy

Study question: Are e-cigarette (e-cig) liquids and nicotine metabolites able to alter the receptivity of stromal endometrial cells (ESC)?
Summary answer: Different e-cigarette liquids and nicotine metabolites affect ESC receptivity and viability with different efficiency by down-regulating implantation marker genes expression and promoting apoptosis of ESC
What is known already: E-cigarettes are instruments that transform a solution of propylene glycol and nicotine in a nicotine-containing aerosol by heating. Studies show that in e-cigarette not-combustion products a number of potentially toxic substances are present. While diseases in heavy cigarette smokers are well documented, the effect of e-cigarettes has been less studied. It has been shown that e-cigaretees may induce acute endothelial dysfunction and promote vascular oxidative stress. Many women are nowadays preferring this way of somoking. However only few works have analyzed effects of e-cigarette on fertility with results that are indicating a possible detrimental effect of these substances on fertility
Study design, size, duration: Endometrium sample biopsies were collected during the proliferative phase of the menstrual cycle from patients undergoing diagnostic hysteroscopy, dissected in smaller pieces then digested with collagenase. Primary cultured ESC were cultured until confluence. ESC were then treated for 1 and 3 hours with different doses of e-cigs liquids or nicotine metabolites (benzo-a-pyrene, cotinine and nicotine ditartrate)
Participants/materials, setting, methods: Primary cultures of endometrial cells were adopted as experimental model (n=7). Treated ESC were processed for total RNA extraction. Gene expression of HOXa10, mucin-1, β3 integrin (implantation marker genes) and FAS (pro-apoptotic gene) normalized by β-actin reference gene, was evaluated by RT-qPCR. Reactions were repeated in triplicate. Cells viability was evaluated by Trypan blue exclusion assay. Statistical analysis was performed with Kruskal-Wallis test followed by the Dunn-Bonferroni's test ($P < 0.05$).
Main results and the role of chance: All the treatment tested showed that e-cigarette liquids and nicotine metabolites are able to significantly down-regulate the expression of implantation marker genes although with different efficiency. E-cig liquids tested (propylene glycol and propylene glycol additioned with 18 mg/ml of nicotine) were all able to down-regulate 1.8-2 folds the HOXa10, mucin-1 and β3 integrin expression when compared to benzo-a-pyrene, cotinine and nicotine ditartate metabolites. E-cigarette liquids increased the relative expression of 2.7 fold of the pro-apoptotic gene FAS if compared to the induction generated by nicotine metabolites. E-cig liquids treatments resulted in higher percentage of cell death after 1 hour incubation (54.4% Vs 31.3%) if compared to nicotine metabolites. After 3 hours of treatments the rate o fcell death was 93.8% for e-cig liquids vs 64.8% for nicotine metabolites.
Limitations, reasons for caution: This study was conducted *in vitro*. Results obtained should be possibly confirmed *in vivo* in the context of the whole human endometrial tissue. Longer incubation to test long-term effects were not possible due to high cell death observed after 3 hours exposure.
Wider implications of the findings: This study demonstrates that e-cigarettes liquids have the same or even higher detrimental effect on the receptivity of endometrium than nicotine metabolites.
Trial registration number: na

P-302 Combined approach to treating endometriosis-associated infertility

N. Artymuk[1], L. Danilova[1], O. Zotova[2]

[1]Kemerovo State Medical University, Obstetrics and Gynecology Department, Kemerovo, Russia C.I.S. ;
[2]Kemerovo Regional Clinical Hospital, Gynecological Department, Kemerovo, Russia C.I.S.

Study question: To comparatively evaluate the effectiveness and compliance of a combined approach to treatment of patients with endometriosis-associated infertility using gestagen and a gonadotropin-releasing hormone agonist.
Summary answer: The use of gestagen and GnRH agonist in the combined therapy of patients with endometriosis-associated infertility and pelvic pain has similar efficacy, but different compliance.
What is known already: Endometriosis affects approximately 10% of reproductive-age women and at least one third of women with infertility. Prevalence of endometriosis has increased up to 50 % in women with infertility. Many studies have now shown that medical treatment for endometriosis does not improve fertility. Hormonal treatment of endometriosis cannot improve fertility and, therefore, their use only leads to delayed pregnancy. In accordance with the recommendations of the CNGOF-HAS, NICE, ESHRE guidelines, suppression of ovarian function should not be prescribed to improve fertility. Nevertheless, a combined approach in the treatment of endometriosis-associated infertility may have advantages in patients with severe pelvic pain.
Study design, size, duration: This was a randomized, open-label, monocentric trial. The study included 150 patients with histologically confirmed endometriosis-associated infertility, by excluding other causes of infertility. Patients from Group I (n=75) after surgical treatment (ablation and/or excision) has been administered the GnRH agonist (buserelin or diphereline) at a dose of 3.75 mg once every 28 days for 3 months. Patients from Group 2 (n=75) prescribed gestagen (dienogest) at a dose of 2 mg daily for 6 months continuously.
Participants/materials, setting, methods: All patients (n=150) underwent laparoscopy and surgical treatment of endometriosis (ablation and / or excision). Almost half of patients with endometriosis-associated infertility from groups I and II (45.3% and 45.3%, respectively) had stages III and IY of the disease. The predominant localization of endometriosis in infertile patients of both groups was the pelvic peritoneum (60%), the sacro-uterine ligaments (88%) and the ovary (44%). After medication therapy, primary and secondary outcomes were assessed.
Main results and the role of chance: The combined treatment of endometriosis-associated infertility via surgery, followed by the administration of both the GnRH agonist and dienogest, was highly similar effective in reducing the symptoms of endometriosis, such as pelvic pain - OR 1,95 [1,01- 3,77] (p=0,051), dysmenorrhea - OR 1,14 [0,56-2,29] (p=0,720), dyspareunia - OR 1,55 [0,77-3,10] (p=0,218), and bleeding - OR 1,44 [0,54- 3,81]. The spontaneous pregnancy rate in both groups was similar, respectively, OR - 0.948 [0.49-1.80] (p = 0.870). The fetal heartbeat registration rate at 12 weeks and live births did not differ statistically significantly between the Groups, however, patients who

were administered aGnRH had a tendency to increase the frequency of non-developing pregnancy - in 17.3% and 9.3% - OR 2.04 [0, 76-5.43], and a lower frequency of live births (p = 0.489). Clinically significant side effects were observed in 92% (69) of patients who were prescribed aGnRH and 16% (12) of women who were administered dienogest - OR 60.375 [21.387-170.441] (p <0.001). The most significant side effects in Group I were: hot flashes (82.7% (62)), decreased libido (74.7% (56)) and sweating (60.0% (45)), in Group II - decreased libido (16 % (12)).

Limitations, reasons for caution: The trial is limited in sample size, single-center, no registration number. Further extensive randomized multicenter studies are required to confirm the benefits of administering gestagens in combination therapy for patients with endometriosis-associated infertility and pelvic pain.

Wider implications of the findings: Both approaches to the management of patients with endometriosis-associated infertility and pain can be successfully used, however, the administration of gestagen has advantages due to the lower incidence of side effects.

Trial registration number: No

P-303 Green tea extract administration reduces MDA level, TNFa expression and endometriotic implants area in mice

Y. Trisetiyono[1], S.T. Hidayat[1], N. Pramono[1], W. Widjiati[2]

[1]Diponegoro University, Department of Obstetrics and Gynecology, Semarang, Indonesia ;
[2]Airlangga University, 2Department of Embryology, Surabaya, Indonesia

Study question: Can the green tea extract (GTE) decrease oxidative stress, inflammation, angiogenesis, and reduce the extent of endometriosis implants in mice?

Summary answer: GTE administration resulted in lower malondialdehyde (MDA), tumor necrosing factor-alpha (TNF-a), and smaller endometriotic implant, but no effect on vascular endothelial growth factor (VEGF).

What is known already: Several studies showed GTE contains catechins, which is a powerful antioxidant, may decrease MDA titer. It also had anti-inflammatory action by regulating the expression of inflammatory cytokines and enzymes. Other studies in mice also found that GTE inhibited microvessel formation in endometriotic implants by selectively suppressing VEGF. Some previous studies also revealed the GTE administration on endometriosis mice induced regression of this disease.

Study design, size, duration: This is an experimental study in mice. The size of the sample was obtained by using the equation of resources formula (21 mice). This research had been done in 3 months (October-December 2017).

Participants/materials, setting, methods: Twenty-one Balb/c female mice were divided into the control (C) group consisted of 7 untreated endometriosis mice model, the second group injected with 1 mg/kg BW leuprolide acetate (LA), and the third group given with 200 mg/kg BW/day GTE. MDA level was measured by spectrophotometry, TNF-a, and VEGF expression by IHC, whereas the area of the endometriotic implants was measured using computerize tracing.

Main results and the role of chance: Compared to MDA serum in the C group, both GTE and LA administration to endometriosis mice resulted in a significantly lower level (p=0.000), but they did not affect to VEGF expression (p=0.123). Only GTE has a significant effect of lowering TNF-a expression compared to the C group (p=0.04). Finally, the area of the endometriotic implants in the GTE and LA groups was smaller than the C group (p=0.008).

Limitations, reasons for caution: The treatment given to each group in different ways that may affect the pharmacological treatment and the body's response of the mice. The study also did not combine GTE and LA, so it did not answer whether GTE can strengthen the therapeutic effect of LA on endometriosis in mice.

Wider implications of the findings: The overall results of this study show that GTE has a good effect on endometriosis disease. Thus, further investigation is needed to be conducted to consider green tea as herbal medicine that can be used as an alternative therapy for endometriosis.

Trial registration number: not applicable

P-304 Autologous platelet-rich plasma optimizes endometrial thickness in women with refractory thin endometrium of varied aetiology during fresh and frozen-thawed embryo transfer cycles

Y. Dogra[1], N. Singh[1]

[1]All India Institute of Medical Sciences, Division of Reproductive Medicine-Department of Obs and Gynaecology, New Delhi, India

Study question: Does platelet-rich plasma (PRP) alters the endometrial milieu making it receptive to an embryo in women with persistent thin endometrium due to varied aetiology?

Summary answer: PRP enhances the endometrial thickness (EMT) significantly in both fresh and FET cycles in thin endometrium associated with tuberculosis(TB), PCOS and Diminished Ovarian Reserve (DOR).

What is known already: Autologous PRP increases endometrial thickness during Frozen thawed Embryo transfer (FET) in women with previous cancelled cycles due to refractory thin endometrium despite hormone replacement therapy (HRT). Limited literature is available regarding the effect of PRP in women with persistent thin endometrium planned for Fresh IVF-ET cycles as well as in thin endometrium due to different etiological factors. The role of PRP specific to underlying etiology of thin endometrium has not been studied earlier.

Study design, size, duration: A prospective interventional cohort study was conducted at Reproductive Medicine Centre at a tertiary care institute. Women with persistent thin endometrium despite HRT were enrolled in the study during a period from December 2018 to December 2019. Eighteen women underwent 21 cycles of intrauterine PRP administration during fresh and frozen embryo transfer cycles. Autologous venous blood was used to prepare PRP using double centrifuge technique .

Participants/materials, setting, methods: Women who were declined fresh IVF-ET cycles because of persistent thin endometrium were selected. PRP was administered during unstimulated cycle on day 8/9 and repeated after 48 hours until ET reached 7 mm. Women who achieved adequate ET were only enrolled. During fresh IVF, if endometrium was inadequate on day 8, PRP was infused and repeated after 48 hours if required. Women with history of prior cancelled cycles were also enrolled for PRP during FET.

Main results and the role of chance: Out of 18 patients, 11 had tubal factor with either hysteroscopic or laparoscopic evidence of TB, 4 patients had DOR, and 3 had PCOS as cause of infertility. Out of 21 PRP cycles in 18 patients, 12 had fresh IVF and 9 had FET. Data obtained was analysed using STATA (version 12.0). Mean age was 31.81±3.5 years. Mean baseline ET was 4.76±0.72 mm. Mean ET after HRT was 5.45±0.53 mm. During Fresh IVF, mean ET before PRP was 5.87 ± 0.86 mm and after PRP was 7.2 ± 0.5mm (t -7.737, df 11, p-value 0.000) with average increase of 1mm. During FET cycles, mean ET before PRP was 5.5±1.02 mm and after PRP was 7.2 ± 0.75mm (t -4.703, df 8, p-value 0.002) with average increase of 0.9mm. There was no significant difference between clinical pregnancy rate (CPR) and implantation rate (IR) in fresh vs FET cycles(25% and 13.7% vs 11.1% and 4.5%, p=0.6). The average increase in ET after first PRP was significant in both TB and DOR (1mm and 1mm, $p < 0.05$) as compared to PCOS (0.7mm, p= 0.07). There was no significant difference in CPR among three etiological factors (23% vs 20% vs 0%, p=0.99).

Limitations, reasons for caution: The limitation of present study is its small sample size. However, the study is currently being endeavoured, and these are the interim results of the same. Further, more extensive studies in broader horizons are required to assess the efficacy of PRP on thin endometrium of various pathophysiologies.

Wider implications of the findings: The present study favours the role of PRP in women who were deferred to undergo IVF or to opt for surrogacy because of their refractory thin endometrium. PRP should be administered to improve the reproductive outcome only in the selected group of population with a persistent thin endometrium.

Trial registration number: CTRI/2018/12/016799

P-305 Microbial roadmap along the menstrual cycle: a new approach to identify active microorganism in the uterus

A. Sola-Leyva[1,2], N.M. Molina[1,2], E. Andrés-León[3], J. Plaza-Díaz[4], M.J. Sáez-Lara[1], J. Fontes-Jiménez[2,5], J. Mozas-Moreno[2,5], B. Romero-Guadix[2,5], S. Ruiz-Durán[2,5], L. Martínez-Navarro[2,5], S. Altmäe[1,2,6]

[1]University of Granada- Faculty of Sciences, Biochemistry and Molecular Biology I, Granada, Spain ;
[2]Instituto de Investigación Biosanitaria de Granada, ibs.GRANADA, Granada, Spain ;
[3]Instituto de Parasitología y Biomedicina "López-Neyra" CSIC, Unidad de Bioinformática, Granada, Spain ;

[4]University of Granada- Faculty of Pharmacy, Biochemistry and Molecular Biology II, Granada, Spain ;
[5]HU Virgen de las Nieves, U. Reproducción- UGC Laboratorio clínico y UGC Obstetricia y Ginecología, Granada, Spain ;
[6]Competence Centre on Health Technologies, Biotechnology Company, Tartu, Estonia

Study question: Does endometrium harbour functionally active microorganisms and whether these microbes differ between proliferative and mid-secretory phases?
Summary answer: Endometrium possesses active microorganisms, and the composition and metabolic function of these microorganisms change along the menstrual cycle.
What is known already: Endometrium harbours its unique microbial composition, however, as all the microbiome studies are based on microbial DNA sequence analysis, there is no knowledge whether these detected bacterial sequences reflect functionally active/alive bacteria within the uterus. Additionally, it is not clear whether there are microbial changes along the menstrual cycle, as a number of studies have detected no microbiome differences between different menstrual cycle phases, while other studies have noticed differences. Thus, whether endometrium harbours alive microbes and whether there are changes along the menstrual phase remains an open issue.
Study design, size, duration: RNA-seq data from 14 endometrial paired samples from healthy women, 7 samples from the mid-secretory phase and 7 samples from the consecutive proliferative phase were analysed for the microbial RNA sequences.
Participants/materials, setting, methods: The raw RNA sequencing data was converted into FASTQ format using SRA Toolkit. In order to eliminate rRNA reads SortMeRNA software was used. R package MetagenomeSeq was used for metataxonomic microorganismal analyses and HUMAnN2 algorithm was used for functional metagenome. The microbial maps were created using Kraken metagenomic sequence classification. With this approach we were able to identify and map the 'active' microorganisms within the samples.
Main results and the role of chance: With our study approach we were able to map the existing functionally active microorganisms in the endometrium along the menstrual cycle. Microbes such as bacteria, fungi, viruses and archaea were identified. At the taxonomical level, after stringent multiple correction, significant differences in the microorganismal composition between proliferative and mid-secretory phases were detected. Further, these microbes were involved in different metabolic functions, where amino acid and carbohydrate metabolisms were more active in the proliferative phase while lipid and energy metabolisms were more active in the secretory phase.
Limitations, reasons for caution: These first pilot study results should be confirmed in a bigger sample size.
Wider implications of the findings: Our study confirms the presence of 'active' bacteria, fungi, viruses and archaea in the human uterus. We detected significant differences in the microbial composition and the involved metabolic functions between the proliferative and mid-secretory phases. Our study findings support the hypothesis that endometrial microbiome plays role in the endometrial functions.
Trial registration number: not applicable

P-306 Heat Shock Protein 90 Inhibitor 17-AAG Inhibits Proliferation of Human Endometriotic Stromal Cells by Repressing Estrogen Receptor Transcriptional Activity.

J. Li[1], P. Chen[2], X. Liang[1]

[1]the Sixth Affiliated Hospital- Sun Yat-sen University, Reproductive Medicine Research Centre, Guangzhou- Guangdong, China ;
[2]the First Affiliated Hospital- Sun Yat-sen University, Department of Pharmacy, Guangzhou, China

Study question: HSP90 is known to be associated with estrogen receptor (ER) and regulates ER mediated cell proliferation. Whether 17-AAG, a HSP90 inhibitor, suppresses the growth of endometriotic cells is still unknown.
Summary answer: 17-AAG could control the progress of endometriotic stromal cells in vitro via inhibiting transcriptional function of the estrogen receptor.
What is known already: The prevalence of endometriosis is between 2% and 10% in the general population and 50% in the infertile population. Current medical treatments such as GnRH agonists and oral contraceptives, are often associated with high recurrence rates and side effects such as hot flushes, episodes of depression and iatrogenically induced osteoporosis. The identification of new compound candidates for endometriosis treatment is needed. Endometriosis is an estrogen-dependent disease. ER is a nuclear receptor that controls the expression of estrogen responsive genes containing estrogen-responsive elements (EREs). Hsp90 is essential for ER binding to the EREs.
Study design, size, duration: Patient recruitment was carried out at the Sixth Hospital of Sun Yat-sen University. Ectopic endometrium (endometriotic tissue, n=12) was collected from patients with ovarian endometriotic cysts undergoing laparoscopy from January 2018 to January 2019.
Participants/materials, setting, methods: Primary cultured human endometriotic stromal cells were seeded and incubated with 10nM, 100nM, 1μM and 10μM 17-AAG for 24 h. Cell viability was tested by MTT assay. Cell proliferation was detected by a colorimetric BrdU incorporation assay. Caspase-3 activity was assessed by Caspase-Glo luminescent-based assays. Transcriptional activity of ER was measured by transient transfection of an ERE-tk-Luc reporter construct that contained the consensus ERE sequences. Luciferase activity was performed to evaluate the activity of ER.
Main results and the role of chance: 17-AAG inhibited the viability of human primary endometriotic stromal cells in a dose-dependent manner from the concentration of 10nM. The growth inhibition by 17-AAG started at 100nM and increased up to 10 μM. The caspase-3 activities were enhanced 2-, 8-, and 11-folds in endometriotic stromal cells after 100nM, 1μM and 10μM 17-AAG treatment for 24h. Similarly, transcriptional activity of ER were inhibited significantly by 17-AAG in a dose-dependent manner.
Limitations, reasons for caution: An experimental mouse model should be established to evaluate the therapeutic effect of 17-AAG. Further molecular mechanism of 17-AAG on ER transcriptional function also need to be explored.
Wider implications of the findings: HSP90 inhibitor controls the development of endometriosis by repressing transcriptional activity of ER. HSP90 inhibitor could provide a valuable tool for better understanding of estrogen signaling, as well as for potential clinical use against estrogen-related diseases of women, including endometriosis.
Trial registration number: not applicable

P-307 Pharmaceutical targets of endometriosis for medical treatment: a systematic review

S.W. Hung[1], R.Z. Zhang[1], C.C. Wang[1]

[1]The Chinese University of Hong Kong, Department of Obstetrics & Gynaecology, Hong Kong, Hong Kong

Study question: Is there any targeted therapy available for treatment of endometriosis?
Summary answer: Approaches through specific-target, multi-targets, or a combination hold their promises to treat endometriosis and manage the complexity of pathophysiology.
What is known already: Growth and development of the endometriotic cells in ectopic sites can be promoted by multiple pathways, including proliferation, anti-apoptosis, migration, invasion, immune escape, inflammation, oxidative stress, angiogenesis, and disrupted autophagy. Endometriosis causes a negative impact on life quality, productivity and infertility. Current medical treatments for endometriosis are rather empirical and incurable. Endometriosis surgery is often performed to improve infertility. Effective medication for specific molecular and signalling pathways is limited.
Study design, size, duration: We systematically reviewed literatures that focused on pharmaceuticals targeting specific molecular and signalling pathways in endometriosis. Selected publications included both animal and human studies; in-vivo or in-vitro. We gathered high quality research studies of 150 publications to summarise the results.
Participants/materials, setting, methods: Literature search was performed using PubMed, Medline and Google Scholar for original articles that were written in English and Chinese. PRISM flowchart and Endnote were used for better management of the searches. We assessed the study quality by critically apprising their appropriateness to the research objective and outcome reproducibility. We presented the data narratively to update the research progress on the medical treatment in endometriosis.

Main results and the role of chance: Akt, ERK, MAPK, MTor, NF-κB, P13K, ROCK, ROS, TNF, Wnt, VEGF, estrogen, cytokines and their upstream and downstream molecules are the key aberrant signalling and regulatory pathways promoting growth and development of endometriotic cells and tissues. Pharmaceuticals targeting these molecules were identified, amongst which Western medicines focus on pain relief and anti-proliferation, such as hormonal (e.g. Dienogest, Aromatase inhibitors) targets on ER stress, estrogen receptors, or TNFα to inhibit NFκB; as well as non-hormonal (e.g. Pentoxifylline, Sunitinib) targets on VEGF and VEGFR for anti-angiogenesis. Natural products and Chinese Medicines aim to reduce chronic pain, resolve infertility and improve quality of life with few side-effects. They mainly regulate blood flow through targeting on superoxide anion and catalase as radical scavengers for anti-oxidation (e.g. EGCG, Glycyrrhizae Radix, Ligusticum Rhizoma) and cytokines and TNFα for anti-inflammation (e.g. Genistein, Angelicae Sinensis Radix, Bupleuri Radix). Chinese Medicine decoctions often contain many individual herbs, so multiple targeting on several pathways with synergistic efficacy and better outcomes.
Limitations, reasons for caution: Non-hormonal treatments are mostly under clinical trials or only studied in in-vitro or animal model. There are lack of experimental and clinical evidences to support its effectiveness and safety of TCM and natural products in alleviating the pathogenesis of endometriosis, when it is compared to western medicine.
Wider implications of the findings: New drugs to treat endometriosis and disease related symptoms and complications, with high efficacy, reduced off targets and other side effects, can be further developed based on knowledge of molecular targets. A detailed protein-protein network helps with target deconvolution for therapeutically known compounds.
Trial registration number: NA

P-308 Do PGT-M patients have a higher prevalence of thin endometrial lining and is this explained by prior prolonged hormonal contraceptive use?

I. Homminga[1], E. Ter Meer[1], H. Groen[1], A. Cantineau[1], A. Hoek[1]

[1]University of Groningen-University Medical Center Groningen, Obstetrics and Gynecology, Groningen, The Netherlands

Study question: Is thin endometrial lining on the day of hCG triggering more prevalent in PGT-M patients than in non-PGT patients, and related to differences in previous hormonal contraceptive use?
Summary answer: Endometrial lining <8mm is more prevalent in PGT-M patients compared to non-PGT patients, and this is related to prolonged hormonal contraceptive use up until IVF-treatment.
What is known already: Thin endometrial lining has been associated with lower pregnancy rates in multiple studies. We seemed to notice an increased prevalence of thin endometrial lining in patients attending our clinic for pre-implantation genetic testing for monogenetic disease (PGT-M). We hypothesized this could be due to a prolonged use of hormonal contraceptives up until the start of the PGT-IVF/ICSI treatment, which is typical for this patient group, as they usually want avoid spontaneous pregnancies. Though it is well known that endometrial thinning occurs during the use of e.g. oral contraceptives, little is known about a possible lingering of this effect after cessation.
Study design, size, duration: A retrospective cohort study was performed, including all PGT-M patients attending the University Medical Centre Groningen, between 2009 and 2018. For each PGT-M patient, two non-PGT IVF/ICSI patients (indication male factor, tubal factor or unexplained subfertility) were included, matched for age and treatment period. Prevalence of thin endometrium (<8mm) on the day of hCG triggering was compared between PGT-M and non-PGT patients. Hormonal contraceptive use (both duration and cessation period) was compared between both groups.
Participants/materials, setting, methods: 122 PGT-M patients and 240 non-PGT patients were included. Only first ovarian hyperstimulation IVF/ICSI cycles were included. Patients with endometriosis, PCOS or uterine abnormalities were excluded. Data on history of hormonal contraceptive use prior to the treatment was obtained from patient questionnaires, both duration (years) and cessation period were included. Cessation categories were <1 year (late cessation) or >1 year (early cessation) before treatment. Endometrial thickness was measured on day of HCG triggering.
Main results and the role of chance: Thin endometrium (< 8 mm) on the day of hCG triggering was found significantly more often in the PGT-M group, compared to the non-PGT group: 32% (95%CI: 23%-40%) versus 11% (95%CI: 7%-15%). As expected, more patients in the PGT-M group ceased their hormonal contraception late: 64% (95%CI: 55%-73%) versus 2% (95%CI: 0%-4%) in the non-PGT group. Average duration of hormonal contraceptive use was longer in the PGT-M group than in the non-PGT group (10,6 years SD 4,3 versus 9,3 years SD 4,5, p=0,02). To determine which factors were significant independent predictors of thin endometrium, a multivariate logistic regression analysis was performed including the following variables: PGT-M yes/no, duration of hormonal contraceptive use (years), late cessation yes/no, age, BMI, parity, previous curettage and number of dominant follicles. Only late cessation (OR: 5,1, 95%CI:1,5 – 16,7) and duration of hormonal contraceptive use (OR 1,1, 95%CI: 1,0-1,3) remained significant independent predictors. The odds ratio for PGT-M to predict a thin endometrium changed from 3.8 (95%CI:2,2-6,7) in an univariable analysis to 1,1 (95%CI: 0,3-3,3) in the multivariable analysis, suggesting that the difference in prevalence of thin endometrium between PGT-M and non-PGT patients could be attributed mainly to differences in prior contraceptive use.
Limitations, reasons for caution: The study is retrospective, using self-reported data. Different types of hormonal contraceptives were reported (e.g. levonorgestrel IUD, oral combined contraceptives) which possibly exert different effects on the endometrium, however, study size was too small to make separate analyses.
Wider implications of the findings: This study provides further insight to a possible contributor to the occurrence of thin endometrium: prior hormonal contraceptive use. Future studies should provide more information on its clinical relevance, to determine whether PGT-M patients can be reassured or should be advised to stop hormonal contraceptives in advance of their treatment.
Trial registration number: not applicable

P-309 High pregnancy rate with customized embryo transfer after endometrial receptivity assessment using a transcriptomic approach in patients candidate for eggs donation program

D. Haouzi[1], F. Entezami[2], A. Ferriere-Hoa[3], C. Mauries[3], A. Gala[3], E. Vintejoux[3], C. Brunet[3], C. Vincens[3], S. Bringer-Deutsch[3], S. Brouillet[3], S. Hamamah[3]

[1]INSERM U1203, IRMB- Hôpital St-Eloi- CHRU Montpellier, MONTPELLIER, France ;
[2]American Hospital of Paris, IVF department, Neuilly-Sur-Seine, France ;
[3]Arnaud de Villeneuve hospital- CHU Montpellier, ART/PGD department, Montpellier, France

Study question: The aim of this study was to optimize pregnancy outcome using customized embryo transfer according to the evaluation of endometrial receptivity of patients included in eggs donation program.
Summary answer: Individual evaluation of endometrial receptivity allows a customized embryo transfer (cET) according the endometrium receptivity day and improves pregnancy outcome in oocyte donation program.
What is known already: Many approaches for human endometrial receptivity including microarray has been previously reported. However, efficiency of the tests according to the clinical results is still debated. The aim of this study is to evaluate the endometrial receptivity using a transcriptomic approach in eggs donation program. This approach consists for screening 11 specific genes of the receptivity window.
Study design, size, duration: Endometrial biopsies are performed during the implantation windows 5-9 days after progesterone administration under hormone replacement therapy. Then, the transfer strategy consists in performing cET of blastocysts according the endometrium receptivity day identified using the Win-Test. Therefore, frozen day 2/3 embryos were transferred 72/48 hours before this specific receptivity day, respectively. When the endometrial sample was defined as non-receptive, a second evaluation was performed later, according to transcriptomic result.
Participants/materials, setting, methods: 45 patients in eggs-donation program due to an advanced age (n=36) or premature ovarian failure (n=9) were included. RNAs from biopsies were extracted and the Win-Test gene expression was assessed by qRT-PCR. Positive pregnancy test was defined as at least two consecutive positive b-hCG serum concentration. Clinical pregnancy, implantation rate and live birth rate were recorded after cET according to the transcriptomic results.
Main results and the role of chance: Analyses of endometrial receptivity status (n=108 biopsies) in patients in oocyte donation program (age mean

± SD: 41.2 ± 3.8 years) revealed a strong inter-patient variability of the moment of the opening of the receptivity window within the implantation window. Majority of patients (84%) present a delay of their receptivity window compared to the classical timing for blastocyst transfer (16% at Pg+5/Pg+6). This delay was mainly between 1 (40%) to 2 days (33%), or more (11%). Then, a cET can to be perform during a subsequent cycle according the endometrium receptivity day and in the respect of the synchronization of the fœto-maternal dialogue. Using this strategy, the positive b-hCG and clinical pregnancy rate per patient were 73.3% and 60%, respectively. The implantation and live birth rates after cET were 50% and 48.9%, respectively.

Limitations, reasons for caution: The benefits of this strategy will be analyzed in a large cohort of patients in eggs/embryos donation program

Wider implications of the findings: This finding demonstrated that customized embryo transfer according to the specific cycle day where endometrium is receptive improves both implantation rate and live birth rate in patients in oocyte donation program.

Trial registration number: ID: NCT04192396

P-310 Increased expression of fucosyltransferase 4 (FUT4) mRNA in eutopic endometrium is a specific and sensitive marker of endometriosis

J. Malejczyk[1], M. Żeberkiewicz[1], A. Hyc[1], A. Iwan[1], A. Zwierzchowska[2], E. Barcz[2], A. Ścieżyńska[1]

[1]Medical University of Warsaw, Department of Histology and Embryology, Warsaw, Poland ;
[2]Medical University of Warsaw, 1st Department of Obstetrics and Gynaecology, Warsaw, Poland

Study question: Are the levels of fucosyltransferase 4 (FUT4) mRNA in the eutopic endometrium associated with endometriosis?

Summary answer: *FUT4* mRNA expression is significantly increased in the eutopic endometrium from women with endometriosis and may serve as a diagnostic marker of endometriosis.

What is known already: Retrograde flow of shed endometrial cell may play a role in development of endometriosis. It is postulated that survival of endometriotic cells in the peritoneum is partially related to their decreased apoptosis and increased invasiveness. In particular, development of endometriotic lesions may be related to epithelial precursor/stem cells characterized by expression of stage specific embryonic antigen 1 (SSEA-1/CD15). Expression of SSEA-1 is related to activity of FUT4.

Study design, size, duration: This control-case study involved endometrial and endometrioid cyst samples collected by aspiration biopsy or laparoscopic surgery from 86 women with and without endometriosis.

Participants/materials, setting, methods: The study was conducted at a university research institution. *FUT4* mRNA were examined in eutopic endometrium tissue samples from 58 women with endometriosis (mean age 32.4 years) and eutopic endometrium from 28 control women (mean age 36 years) by means of quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Main results and the role of chance: Expression of *FUT4* mRNA in eutopic endometrium from women with endometriosis and controls did not differ in relation to phase of the menstrual cycle. The relative levels of *FUT4* mRNA were significantly increased in eutopic endometrium from endometriosis patients as compared to control women ($P<0.0001$). ROC analysis of *FUT4* mRNA expression in eutopic endometrium showed high statistical significance (AUC = 0.90, 95%CI = 0.81-0.99, $P<0.0001$) thus indicating endometrial *FUT4* mRNA to be a specific marker of endometriosis. Expression of FUT4 mRNA in eutopic endometrium was correlated with endometriosis severity ($r_s=0.5579$, $P<0.0001$). There were no differences in endometrial *FUT4* mRNA expression regarding endometriotic lesion location and patients' infertility status.

Limitations, reasons for caution: The study was performed on a limited group of patients and controls. The results should be confirmed by a replicate study on different patients' cohorts.

Wider implications of the findings: The present results strongly suggest that expression of *FUT4* mRNA in eutopic endometrium is a specific marker suitable for the low-invasive diagnosis of endometriosis. It is possible that FUT4 activity plays a role in development and persistence of endometriotic lesions and may potentially be a therapeutic target for endometriosis.

Trial registration number: not applicable

P-311 Expression of non-classical Human Leukocyte Antigen (HLA)-G and -F in the endometrium in relation to immune cell infiltration in women experiencing recurrent implantation failure (RIF)

H. Papuchova[1,2,3], M. Hviid Saxtorph[2,4], T. Hallager[2,5], I. Engberg Jepsen[2,4], G. Persson[1,2], J.O. Eriksen[2,5], L. Larsen Grupe[2,5], N. Macklon[2,6], T.V. Hviid[1,2,3]

[1]Zealand University Hospital, Department of Clinical Biochemistry- Centre for Immune Regulation and Reproductive Immunology CIRRI, Roskilde, Denmark ;
[2]Zealand University Hospital, The ReproHealth Research Consortium, Roskilde, Denmark ;
[3]University of Copenhagen, Department of Clinical Medicine, Copenhagen, Denmark ;
[4]Zealand University Hospital, Department of Gynaecology- Obstetrics and Fertility, Roskilde/Køge, Denmark ;
[5]Zealand University Hospital, Department of Pathology, Roskilde, Denmark ;
[6]London Women's Clinic, London Women's Clinic, London, United Kingdom

Study question: Does HLA-F and HLA-G expression correlate with the infiltration of CD56+, CD16+, FOXP3+ or CD163+ immune cells in endometrial biopsies from women with RIF?

Summary answer: Endometrial HLA-G expression of women experiencing RIF is correlated with immune cell infiltration (CD16+ and CD56+). HLA-F expression is correlated with anti-inflammatory macrophage infiltration (CD163+).

What is known already: HLA class Ib have a prominent immunomodulatory role at the fetal-maternal interface, promoting immune tolerance of fetal antigens. HLA-G-positive trophoblasts are in a close contact with decidualized endometrium during pregnancy, and HLA-G expression in the endometrium has been identified as prognostic for achieving pregnancy. Evidence of HLA-F expression in the endometrium and its interactions with immunoglobulin-like transcript (ILT) receptors expressed on Natural Killer (NK) cells, T cells and macrophages have been elucidated. These interactions between endometrial HLA class Ib and immune cells could have a determinant immunosuppressive function required for successful embryo implantation.

Study design, size, duration: Endometrial biopsies were obtained from all participants in an estrogen/progesterone substituted cycle. Samples were collected after five days of progesterone treatment. In total, 86 women with RIF and 37 controls attending a fertility clinic were recruited between November 2017 and September 2019. The patients were divided into four groups based on the infertility cause: unknown, female factor, male factor and both male and female factor. Preliminary data are presented including 51 women experiencing RIF.

Participants/materials, setting, methods: Immunohistochemical procedures were automatically performed on an Autostainer on paraffin-embedded sections using anti-human antibodies for HLA-F, HLA-G, CD56, CD16, CD163 and FOXP3 with corresponding technical staining controls. Randomized and blinded analysis were performed using Tissue IA image analysis software (Digital Image Hub). Algorithms were developed for the above-mentioned antibodies. HLA-F and HLA-G expression were studied by flow cytometry on fresh endometrial samples. Statistical analysis based on Spearman correlation was performed using SPSS version 25.

Main results and the role of chance: HLA-F and HLA-G membrane expression on endometrial cells were confirmed by flow cytometry analysis on freshly isolated endometrial stromal cells from endometrial biopsies. Using immunohistochemistry, the preliminary results show that the percentage of HLA-G-positive cells in the endometrial glands was positively correlated with the percentage of CD16-positive cells per analyzed area ($p<0.05$). As well as the semi-quantitative HLA-G H-score (staining intensity score) was positively correlated with percentage of CD16-positive cells within the analyzed area ($p<0.05$). Moreover, in a patient subgroup with no female factor identified, both percentage of HLA-G-positive cells and HLA-G H-score were positively correlated with percentage of CD16-positive, CD56-positive and CD163-positive cells per analyzed area (all $p<0.05$). Interestingly, all three HLA-F variables (number of positive cells per mm^2, percentage of positive cells per analyzed area and H-score) in both endometrial glands and stroma were positively correlated with anti-inflammatory macrophages per mm^2 and their percentage per analyzed area (identified by CD163, $p<0.01$ and $p<0.005$ for glands and stroma respectively). The number of HLA-F-positive cells per mm^2 and HLA-F score were also negatively correlated with the number of regulatory T cells per mm^2 (identified by FOXP3, all $p<0.05$).

Limitations, reasons for caution: The data presented are preliminary data based on 51 women out of 123 included in the study. Correlations indicate the relationship between two variables; however, influences of other variables should be taken into consideration. No corrections for multiple comparisons were performed because the character of the study is primarily exploratory.
Wider implications of the findings: In the current preliminary analysis, we show interesting trends indicating links between NK cell, regulatory T cell and macrophage markers with HLA class Ib molecules of potential importance for establishing fetal-maternal tolerance at implantation and in early pregnancy.
Trial registration number: not applicable

P-312 16s sequencing of vaginal microbiota in patients undergoing frozen embryo transfer following IVF

K. Neumann[1], A. Masuch[2], S. Graspeuntner[3], L. Kirchhoff[3], A. Schultze-Mosgau[2], M. Depenbusch[2], J. Rupp[3], G. Griesinger[2]

[1]University Hospital of Schleswig-Holstein- Luebeck- Schleswig-Holstein- Germany, Department of Gynecological Endocrinology and Reproductive Medicine, Lübeck, Germany ;
[2]University Hospital of Schleswig-Holstein- Luebeck- Schleswig-Holstein- Germany, Department of Reproductive Medicine and Gynecological Endocrinology, Luebeck, Germany ;
[3]University Hospital of Schleswig-Holstein- Luebeck- Schleswig-Holstein- Germany, Department of Infectious Diseases and Microbiology, Luebeck, Germany

Study question: Does 16s sequencing of vaginal microbiota predict the outcome of a frozen embryo transfer cycle? Does the phase of the menstrual cycle impact these microbiota?
Summary answer: Vaginal microbiota can contribute to pregnancy prediction which seems to be independent from the phase of the menstrual cycle during which sampling was done.
What is known already: Recent studies have shown conflicting data on an association of certain vaginal microbiota with the outcome of a fresh cycle of in vitro fertilization (IVF) (Koedooder et al. 2019, Haahr et al. 2019). These studies, however, analyzed fresh embryo transfer cycles only and did not account for intra-individual variation, e.g. the impact of the sampling time-point within the menstrual cycle.
Study design, size, duration: Prospective, clinical cohort study of patients undergoing a frozen embryo transfer cycle (spontaneous or programmed) following IVF. Sample collection has started in 5/2018 and is still ongoing. The study was prospectively registered (clinicaltrials.gov ID: NCT03507673).
Participants/materials, setting, methods: Vaginal swabs were collected on day of planning of embryo transfer (in the late follicular phase day 13 – 15 of the menstrual cycle), on day of embryo transfer (i.e. after 2 to 6 days of progesterone administration) and on day of pregnancy test (10 – 14 days after embryo transfer). 16s ribosomal RNA sequencing was used for longitudinal investigation of vaginal microbiota which were classified according to previous reported outcome criteria.
Main results and the role of chance: 16s sequencing of the first collected sample from the end of the follicular phase (n=56) of the frozen embryo transfer cycle revealed a statistically significant reduction in species richness (p=0.011) and in the chao 1 index (p=0.0045) for patients achieving a positive pregnancy test (n=22) versus patients having a negative test (n=34). In patients having a sample available for analysis from all three time-points (n=13), a tendency towards an increased relative read count for Lactobacillus crispatus for patients achieving a positive pregnancy test vs. patients having a negative test was observed. Additionally, a decreased alpha-diversity was observed for patients with a positive pregnancy test. These differences were observed independent from the phase of the menstrual cycle during which sampling was performed; however, statistical significance was not reached yet for this subgroup. Surprisingly, n=3 asymptomatic patients undergoing infertility treatment were diagnosed with a relative chlamydial abundance. None of these patients having a positive relative chlamydia read could achieve a pregnancy.
Limitations, reasons for caution: This study is ongoing, a higher number of patients are needed for robust conclusions. Additionally, no stratification of subgroups (e.g. age, protocol of frozen embryo transfer cycle, etc.) or differentiation of previously reported community state types of bacteria have been conducted so far.
Wider implications of the findings: Vaginal microbiota seems to be associated with the outcome of a frozen embryo transfer cycle. This association might be independent from the phase of the menstrual cycle. Asymptomatic chlamydial infections detected by 16s sequencing in patients undergoing IVF needs further investigation.
Trial registration number: clinicaltrials.gov ID: NCT03507673).

P-313 Prevalence of T-shape uterus among porous women based on Congenital Uterine Malformations by Experts (CUME) criteria

A. Seyhan[1], S. Ertas[1], B. Urman[1]

[1]American Hospital, Reproductive Endocrinology and Infertility, Istanbul, Turkey

Study question: What is the prevalence of T-shape uterus among parous women based on CUME criteria?
Summary answer: Parous women who conceived spontaneously have a low prevalance of T-shape uterus.
What is known already: Both prevalence of T shape uterus in parous women and its effect on reproductive/obstetric outcome is unknown.
Study design, size, duration: Prospective cohort study including 238 reproductive age group of women in who conceived spontaneously and had at least one live birth. Participants were recruited from a contraception clinic between January 2017 and December 2019.
Participants/materials, setting, methods: Participants underwent 3D transvaginal ultrasound in the late luteal phase of menstrual cycle. The ESHRE/ESGE classification of congenital anomalies of the female genital tract was used for the description of abnormal findings. The diagnosis of T-shape uterus was based on the definition ofCUME criteria.The following measurements were taken In the coronal view; 1)lateral indentation depth, 2) lateral indentation angle, 3) T-angle. Uterine cavity volume was measured by Virtual Organ Computer-aided AnaLysis (VOCAL™) program.
Main results and the role of chance: The mean (± standard deviation) age was 35.7±5.9 years. Mean parity was 1.88 (min 1 max 7) and time to pregnancy (TTP) was between 1- 12 months. Mean antral follicle count was 12.2±9.5 and 10.5 % had at least one ultrasound feature of adenomyosis. Nine patients (3%) were diagnosed with congenital uterine abnormality of which 5 (2.1%) had partial septate uterus, 2 (0.8%) had a hemiuterus and 1 (0.4%) had a Tshape uterus and 1 had borderline T-shape uterus.

Overall mean uterine volume, lateral indentation depth, lateral indentation angle and T-angle was 4 ±2.3 ml, 2.95±,1.16mm, 154.7 ±9.9°and 73.5±9.9°respectively. Six women fulfilled one criterion and 1 patient met 2 criteria with lateral indentation depth of 8.4mm and lateral indentation angle of 128°. The women with T shaped uterus had uterine volume, lateral indentation depth, lateral indentation angle and T-angle of 2.62 ml, 7mm, 124°and 38°respectively. Her TTP was 7 months and she had given birth to a 3450 gr child at 39.
Limitations, reasons for caution: The prevalence of T-shape uterus in a population of parous women who conceived spontaneously is very low. The management of this condition can be determined following comparative studies showing the prevalance among parous, recurrent miscarriage and infertilite patients.
Wider implications of the findings: If women with infertility and recurrent misccariage are shown to have higher prevalance of T shape uterus, possible benefit of surgical management may be claimed.
Trial registration number: 2018.052.IRB1.008

P-314 Is endometrial gene expression profile affected in women with endometriosis? a meta-analysis

E. Vargas[1], E. García-Moreno[2], A. Salumets[3], F.J. Esteban[1], S. Altmäe[4]

[1]University of Jaen, Department of Experimental Biology, Jaen, Spain ;
[2]Puerta del Mar University Hospital, Immunology Unit, Cadiz, Spain ;
[3]Competence Centre on Health Technologies, Competence Centre on Health Technologies, Tartu, Estonia ;
[4]University of Granada, Department of Biochemistry and Molecular Biology, Granada, Spain

Study question: Do women with endometriosis have a different endometrial gene expression profile at the time of embryo implantation than healthy women?

Summary answer: This meta-analysis identified no single genes to be differentially regulated in endometriosis, while apoptosis, immune response and wound healing pathways seem to be dysregulated.
What is known already: ~50% of women with endometriosis suffer from infertility. Some studies suggest impaired endometrial receptivity in these women,while other studies claim no impact of endometriosis in endometrial receptivity. The studies analysing endometrial gene expression profiles in endometriosis have been performed in small sample size with low statistical power. We set out to systematically gather published studies comparing endometrial gene expression signatures in women with endometriosis vs. healthy women. Based on the obtained data we conducted meta-analysis of differentially expressed genes in order to raise the analysis power and thus clarify whether endometriosis have an impact on endometrial receptivity at molecular level.
Study design, size, duration: A systematic literature search was performed until 16th of January 2020 aiming to identify studies assessing the mid-secretory endometrial gene expression profile, i.e. transcriptome of women with endometriosis vs. healthy controls during the window of implantation. The eligible studies were further explored to extract the differentially expressed gene lists and the associated fold changes and we then performed a meta-analysis using the robust rank aggregation (RRA) method to screen for highly representative genes.
Participants/materials, setting, methods: The systematic literature search was conducted following the PRISMA criteria and included Pubmed, Cochrane, Google Scholar and Web of Science. A meta-analysis was performed on the selected studies to extract the differentially expressed genes described during the mid-secretory phase in women with endometriosis vs. healthy women using the RRA method. In total endometrial transcriptome data of 128 women (84 patients and 44 controls) were analysed in our meta-analysis.
Main results and the role of chance: Within the systematic literature search, 7 studies were eligible for the quantitative meta-analysis gathering transcriptome data in total from 128 women of the mid-secretory phase of the menstrual cycle. It is the first time when such a number of women have been analysed in order to assess the impact of endometriosis on endometrial transcriptome profiles. The transcriptome data from 128 women covered a total number of 1100 differentially expressed transcripts (644 up-regulated and 456 down-regulated), whose relevance was interrogated using the RRA method. After stringent multiple correction, our meta-analysis did not detect any significantly differentially expressed genes in the mid-secretory endometrium of patients vs. controls. However, when analysing the molecular pathways involved in the identified gene lists, we found that apoptosis, immune response and wound healing pathways were dysregulated among women with endometriosis.
Limitations, reasons for caution: Most of the studies included into the meta-analysis are relatively small, using different platforms for measuring gene expression together with the low number of samples which might contribute to a bias.
Wider implications of the findings: The current meta-analysis supports the hypothesis that a single gene is not dysregulated in endometriosis, rather is a cohort of genes that lead to dysregulation of specific molecular pathways.
Trial registration number: NA

P-315 The intra- and interobserver reproducibility of pelvic ultrasound for the detection and measurement of endometriotic lesions

E. Bean[1], P. Chaggar[1], N. Thanatsis[1], W. Dooley[1], C. Bottomley[1], D. Jurkovic[1]

[1]University College London Hospital, Women's Health, London, United Kingdom

Study question: What is the interobserver and intraobserver reproducibility of pelvic ultrasound for the detection of endometriotic lesions?
Summary answer: Pelvic ultrasound is highly reproducible for the detection of pelvic endometriotic lesions
What is known already: Transvaginal ultrasound has been widely adopted as the first-line assessment for the diagnosis and assessment of pelvic endometriosis. Severity of endometriosis as assessed by ultrasound has been shown to have good concordance with laparoscopy (kappa 0.79). The reproducibility of transvaginal ultrasound for assessment of ovarian mobility and pouch of Douglas obliteration using the 'sliding sign' has already been described in the literature. However, there is no available data in the literature to demonstrate the intraobserver repeatability of measurements for endometriotic cysts and nodules.
Study design, size, duration: This was a prospective observational cross-sectional study conducted over a period of 12 months. We included 50 consecutive women who were all examined by two operators (A and B) during their clinical attendance.
Participants/materials, setting, methods: The study was carried out in a specialist endometriosis centre. We included all consecutive women who had ultrasound scans performed independently by two experienced operators during the same visit to the clinic. The outcomes of interest were the inter- and intraobserver reproducibility for the detection of endometriotic lesions. We also assessed repeatability of the measurements of lesion size.
Main results and the role of chance: There was a good level of agreement between operator A and operator B in detecting the presence of pelvic endometriotic lesions (k = 0.72). There was a very good level of agreement between operators in identifying endometriotic cysts (k = 0.88) and a good level of agreement in identifying endometriotic nodules (k = 0.61). The inter- and intraobserver repeatability of measuring endometriotic cysts was excellent (intraclass correlation (ICC) ≥ 0.98). There was good interobserver measurement repeatability for bowel nodules (ICC 0.88), but the results for nodules in the posterior compartment were poor (ICC 0.41). The intraobserver repeatability for nodule size measurements was good for both operators (ICC ≥ 0.86).
Limitations, reasons for caution: There was insufficient data to perform a separate analysis for nodule size in the anterior compartment. All examinations were performed within a specialised unit with a high prevalence of deep endometriosis. Our findings may not apply to operators without intensive ultrasound training in the diagnosis of pelvic endometriosis.
Wider implications of the findings: These findings are important because ultrasound has been widely accepted as the first line investigation for the diagnosis of pelvic endometriosis, which often determines the need for future investigations and treatment**.** The detection and measurement of bowel nodules is essential for anticipation of surgical risk and planning surgical excision.
Trial registration number: Not applicable

P-316 Incidence of the Low Anterior Syndorme (LARS) after Colorectal Surgery for Deep Endometriosis: an International Multicentric Study

A. Bokor[1], G. Hudelist[2], B. Dauser[3], R. Brubel[1], J.J. Tuech[5], H. Roman[4]

[1]Semmelweis University, Dept.OB/GYN, Budapest, Hungary ;
[2]Hospital St. John of God Vienna- Austria, Gynecology, Vienna, Austria ;
[3]Hospital St. John of God- Vienna- Austria, Department of General Surgery, Vienna, Austria ;
[4]Centre d'endométriose Clinique Tivoli-Ducos Bordeaux- France, Endometriosis Centre, Bordeaux, France ;
[5]Department of surgery, Rouen University Hospital F-76000, France

Study question: Is there a difference between the incidence of the Low Anterior Syndrome (LARS) after segmental resection or transanal disc excision performed for the treatment of colorectal deep endometriosis (DE).
Summary answer: LARS is not more frequent after nerve and vessel spearing segmental resection (NVSSR) when compared to a more conservative approach, the Rouen-technique disc excision.
What is known already: Several studies show a significant drop in pain scores and improved fertility outcomes in women following surgical resection of colorectal endometriosis. However, there is inceasing evidence that intermediate and long-term bowel dysfunction may occur as a consequence of radical surgery for rectal DE. Typical symptoms include constipation, feeling of incomplete evacuation, clustering of stools and urgency. This is described in the colorectal surgical literature as LARS. Previous studies demonstrated statistically significant differences in functional outcomes in favour of the conservative surgical approach, i.e. resection of endometriotic tissue with preservation of the luminal structure of the rectal wall.
Study design, size, duration: A total of 205 patients, operated before the 1st of January 2018, were enrolled in this study. One hundred and thirty-nine patients with nerve and vessel sparing segmental resection (NVSSR) from the University Hospital Rouen, France (n=52); Semmelweis University Budapest, Hungary (n=50) and Hospital of St.John of God Vienna, Austria (n=37) as well as 66 patients operated at the University Hospital Rouen, France using the Rouen-technique disc excision (RTDE) were included.
Participants/materials, setting, methods: All patients underwent low rectal resection with a resection line lower than 7cm from anal verge.

Gastrointestinal functional outcomes of the two procedures were compared using the validated LARS questionnaire.

The median follow-up time was 46±11 months. As a secondary outcome, the surgical sequelae and fertility results were examined. Statistical analysis was done using Fisher's Exact and Pearson Chi Square test.

Main results and the role of chance: We found no statistically significant difference between the incidence of the LARS (31.7% and 37.9% respectively) among patients operated using RTDE when compared to women treated by NVSSR (p=0.4). The occurence of LARS was positively associated with the use of protective ileo-or colostomy (p=0.02). A higher rate of severe complications was observed in RTDE (19.7%) compared to patients who underwent NVSSR (9.0%, p=0.029).

Limitations, reasons for caution: Since digestive complaints may also be present presurgically, evaluation of digestive complaints should have been recorded pre- and post surgery as well.

Although this study has important limitations due to its retrospective design, it highlights important factors that affects outcomes in patients undergoing colorectal resection for the treatment of DE.

Wider implications of the findings: LARS is not more frequent after NVSSR when compared to a more conservative approach RTDE.

The findings of this study may help guide treatment strategies aimed at providing patients with colorectal DE an optimal surgical therapy. To confirm our findings prospective studies are required.

Trial registration number: NA

P-317 Increased rate of spontaneous miscarriages in women affected with adenomyosis associated with endometriosis

B. Pham[1], M. Bourdon[1], L. Marcellin[1], C. Maignien[1], C. Chapron[1], P. Santulli[1]

[1]Hopital Cochin Port-Royal, Gynecology and reproductive medecine, Paris, France

Study question: Among women with a radiological diagnosis of adenomyosis, are spontaneous miscarriages higher in women with endometriosis when compared with endometriosis-free controls?

Summary answer: Endometriosis-affected women display a significantly higher rate of previous spontaneous miscarriages than endometriosis-free controls in a population of adenomyosis affected women.

What is known already: Endometriosis and adenomyosis are common, estrogen-dependent, inflammatory disorders in women of reproductive age. Those diseases could be responsible for pain and fertility alteration. Endometriosis and adenomyosis are associated in a large part of women. In endometriosis, an association with an increased incidence of miscarriage was demonstrated compared to endometriosis-free women. The association between adenomyosis and miscarriages has also been shown. When endometriosis and adenomyosis are associated, we do not know what is their share of responsibility in the occurrence of miscarriages.

Study design, size, duration: An observational study nested in a cohort of women aged between 18-42 years who had undergone surgical exploration for benign gynecological conditions or who underwent assisted-reproductive-technology at our institution between May-2005 and May-2018. Only women who presented adenomyosis lesions visualized at uterine-MRI were retained for this study. Women who had never been pregnant before were excluded. The control-group included patients presenting adenomyosis without lesions of endometriosis and the study-group patients with adenomyosis associated with endometriosis.

Participants/materials, setting, methods: Data were collected preoperatively using a structured questionnaire. Among women who previously conceived, the type and number of the different previous first trimester pregnancies outcomes were studied. Previous history of miscarriage was studied according to the phenotype of adenomyosis (focal and/or diffuse lesions) and the phenotype of endometriosis (superficial, ovarian and/or deep infiltrating lesions).

Main results and the role of chance: 214 pregnancies in 'Adenomyosis associated with endometriosis' group and 53 pregnancies in 'Adenomyosis-affected women without endometriosis' group were analysed. Previous miscarriage rate was significantly higher in adenomyosis affected women with endometriosis compared to the controls (61/214 [28.5%] vs. 6/53 [11.3%], respectively; p = 0.009). After multivariable generalized estimating equation logistic regression model, adjusted on adenomyosis and endometriosis phenotypes, the association with endometriosis in women affected with adenomyosis significantly increase the risk of miscarriage (OR=3,198, 95% CI [1,1-9,65]). The risk was significantly higher in case of deep infiltrated endometriosis (OR 4,37, 95% CI [1,32-14,53]).

Limitations, reasons for caution: The exclusive inclusion of patients from our referral center could constitute a possible selection bias, as those women may have suffered from particularly severe forms of adenomyosis and/or endometriosis.

Wider implications of the findings: We reported in this study that endometriosis associated with adenomyosis is associated with higher previous spontaneous miscarriage rate as compared to women with isolated adenomyosis. This study opens the doors to future, more mechanistic studies to establish the exact link between endometriosis/adenomyosis and spontaneous miscarriage rates.

Trial registration number: not applicable

P-318 Alteration in gene expression of H19 lncRNA and IGF2 in endometrial tissues of women with endometriosis

S. Kamrani[1,2], E. Amirchaghmaghi[3,4], K. Ghaedi[1,5], M. Shahhoseini[2,6,7]

[1]Department of Cell and Molecular Biology and Microbiology- Faculty of Biological Science and Technology- University of Isfahan- Isfahan- Iran., Department of Cell and Molecular Biology and Microbiology, Isfahan, Iran ;
[2]Department of Genetics- Reproductive Biomedicine Research Center- Royan Institute for Reproductive Biomedicine- ACECR- Tehran- Iran., Department of Genetics, Tehran, Iran ;
[3]Department of Regenerative Biomedicine- Cell Science Research Center- Royan Institute for Stem Cell Biology and Technology- ACECR- Tehran- Iran., Department of Regenerative Biomedicine- Cell Science Research Center-, Tehran, Iran ;
[4]Department of Endocrinology and Female Infertility- Reproductive Biomedicine Research Center- Royan Institute for Reproductive Biomedicine- ACECR- Tehran- Iran., Department of Endocrinology and Female Infertility- Reproductive Biomedicine Research Cent, ;
[5]Department of Cellular Biotechnology- Cell Science Research Center- Royan Institute for Biotechnology- Isfahan- Iran., Department of Cellular Biotechnology- Cell Science Research Center, Isfahan, Iran ;
[6]Department of Cell and Molecular Biology- School of Biology- College of Science- University of Tehran- Iran., Department of Cell and Molecular Biology- School of Biology, Tehran, Iran ;
[7]Department of Reproductive Epidemiology Research Center- Royan Institute for Reproductive Biomedicine- ACECR- Tehran- Iran, Department of Reproductive Epidemiology Research Center, Tehran, Iran

Study question: Is gene expression profile of *H19 lncRNA* and *IGF2* different in endometrial tissues of women with endometriosis in compare to normal endometrium?

Summary answer: Decreased *H19* and *IGF2* expression was showed in eutopic and ectopic endometriual tissues of women with endometriosis.

What is known already: Endometriosis is one of the most common diseases in female reproductive system. Several factors, including genetic and epigenetic factors are suggested to be involved in its pathogenesis. Adhesion and proliferation of endometrial tissue, are essential elements in the pathogenesis of endometriosis. Thus growth factors such as insulin-like growth factor-2 (IGF2) may be involved as inducers of cellular proliferation. *IGF2* gene is among the most regulated genes in endometriosis that play important roles in regulating growth and differentiation of endometrial cells. Another factor is imprinted lncRNA H19. It is known to be involved in the regulation of cell proliferation and differentiation.

Study design, size, duration: In this case-control study, 5 endometriotic lesions (ectopic), 5 endometrial samples (eutopic) of women with endometriosis and 10 endometrial control samples were analysed. Control samples were obtained from women who had no evidence of endometriosis during laparoscopy in Royan Institute. Ectopic samples were obtained during laparoscopy surgery. Control and eutopic endometrial samples were obtained by pipelle. All women signed the informed consent form and did not receive any hormonal treatments during the last three months.

Participants/materials, setting, methods: After endometrial tissues collection, RNA extraction and cDNA synthesis were done. Real-time PCR

technique was used for quantitative gene expression of H19 lncRNA and IGF2. Gene expression data were analyzed based on $2^{-\Delta\Delta CT}$ to estimate the relative fold change value. One-Way ANOVA was used for data analysis. P value less than 0.05 was considered statistically significant.
Main results and the role of chance: Gene expression profile of H19 and IGF2 was decreased in eutopic and ectopic endometrial lesions compared with control samples. These decreases were not statistically significant (p>0.05). In addition, gene expression levels of these genes were lower in ectopic lesions in compare to eutopic samples in endometriosis women. These differences were not significant.
Limitations, reasons for caution: The main limitations of this study is the small sample size. For getting more information, we are going to study these genes in a large number of women with and without endometriosis.
Wider implications of the findings: It seems altered gene expression of H19 and IGF2 could play role in the development of endometriosis, because of their roles in regulatory pathways of cellular proliferation. After increasing sample size, epigenetic analysis will be performed on the samples.
Trial registration number: -

P-319 Increased expression of CREM is associated with decreased binding of its inhibitor (ICER) to the promoter region of CYP19 aromatase gene in endometriosis

S. Kalantari[1,2], A. Saadat Varnosfaderani[1,2], F. Ramezanali[3], E. Amirchaghmaghi[3,4], M. Shahhoseini[5,6,7]

[1]Reproductive Biomedicine Research Center- Royan Institute for Reproductive Biomedicine- ACECR., Department of Genetics, Tehran, Iran ;
[2]Faculty of Basic Sciences and Advanced Technologies in Biology- University of Science and Culture, Department of Cell and Molecular Biology Science, Tehran, Iran ;
[3]Reproductive Biomedicine Research Center- Royan Institute for Reproductive Biomedicine, Department of Endocrinology and Female Infertility, Tehran, Iran ;
[4]Cell Science Research Center- Royan Institute for Stem Cell Biology and Technology- ACECR, Department of Regenerative Biomedicine, Tehran, Iran ;
[5]Reproductive Biomedicine Research Center- Royan Institute for Reproductive Biomedicine- ACECR, Department of Genetics, Tehran, Iran ;
[6]School of Biology- College of Science- University of Tehran, Department of Cell and Molecular Biology, Tehran, Iran ;
[7]Royan Institute for Reproductive Biomedicine- ACECR, Reproductive Epidemiology Research Center, Tehran, Iran

Study question: Is there any association between gene expression of CREM and binding of its inhibitory isoform ICER to CYP19 promoter II (PII) in women with endometriosis?
Summary answer: Overexpression of CREM was detected in the ectopic endometrium and the binding of ICER to CYP19 promoter II in eutopic tissues was decreased.
What is known already: Endometriosis is an estrogen-dependent disease. The synthesis of estrogen depends on the expression of the aromatase enzyme (CYP19 gene). cAMP response element modulator (CREM) is a member of the bZIP family of transcription factors that has an important role in the regulation of aromatase gene expression. Inducible cAMP early repressor (ICER) is one of CREM inhibitory isoforms that represses cAMP-induced transcription.
Study design, size, duration: Endometriotic biopsies (ectopic), endometrial samples (eutopic) of women with endometriosis and endometrial control samples were collected. Control samples were obtained from women who had no evidence of endometriosis during laparoscopy. Ectopic biopsies from endometriosis women were obtained through laparoscopy while eutopic samples and control endometrial samples were collected by pipelle. Twelve endometrial samples from each group were used for gene expression analysis of CREM, while 6 control and 5 eutopic samples used for ChIP assay.
Participants/materials, setting, methods: Total RNA extraction and cDNA synthesis were done. Real-time PCR technique was used for quantitative gene expression of CREM. One-way ANOVA was used for data analysis. For protein-DNA interaction analysis, soluble chromatin was extracted and chromatin immunoprecipitation (ChIP) coupled with Real-time PCR was performed to quantify the binding of ICER to CYP19 promoter II in control and eutopic endometrial samples.
Main results and the role of chance: Gene expression level of CREM was statistically increased in ectopic lesions compared with control (P=0.001) and eutopic (P=0.007) samples. Increased expression of the CREM gene was also detected in eutopic endometrial samples compared to controls, while this difference was not statistically significant (P>0.05). In addition, the binding of ICER to CYP19 promoter II was statistically decreased in eutopic samples compared to the control endometrial group (P=0.005).
Limitations, reasons for caution: Larger sample size for the confirmation of these data is needed. Epigenetic evaluation of ectopic endometrial tissues especially in CYP19 promoter II is recommended.
Wider implications of the findings: The overexpression of CREM in the endometriosis tissue samples and decreased binding of the inhibitory isoform of CREM (ICER) to the CYP19 prompter II may contribute to the pathogenesis of endometriosis via its regulatory role in the expression of estrogen biosynthesis enzymes.
Trial registration number: not applicable

P-320 Oral dydrogesterone compared to intravaginal micronized progesterone for endometrial preparation in frozen-thawed embryo transfer cycles: preliminary results of a randomized controlled trial

M. Cavagna[1], L.C. Mattos de Macedo[1], S. Rolim[2], A. Dzik[1], N. Donadio[1], H.S. Marçon[1], M.D. Vasconcelos[1], B. Merquires de Araujo[1], S.M. Franceschini Cinquetti[1], A. Mantovani Fonseca[1], A.R. Rocha[1]

[1]CRSM Hospital Perola Byington, Human Reproduction, Sao Paulo, Brazil ;
[2]Faculdade de Ciências Médicas da Santa Casa de Sao Paulo, Obstetrics and Gynecology, Sao Paulo, Brazil

Study question: Can we achieve similar reproductive outcomes assessed by ultrasound scan at 12 weeks of gestation using oral dydrogesterone or vaginal micronized progesterone for endometrial preparation in frozen-thawed embryo transfer (FET)?
Summary answer: At present, our preliminary results are suggesting that reproductive outcomes of FET are similar with both methods of progesterone supplementation (oral dydrogesterone versus vaginal progesterone).
What is known already: In recent investigations, it has been demonstrated that oral dydrogesterone for luteal phase supplementation in fresh embryo transfer is as effective as micronized progesterone regarding reproductive outcomes. Nevertheless, nothing is known about FET cycles.
Study design, size, duration: We are performing a prospective randomized study comparing the use of oral dydrogesterone and intravaginal micronized progesterone for endometrial preparation in FET cycles.
Participants/materials, setting, methods: So far, 44 patients were randomly selected to receive either oral dydrogesterone, 40 mg/daily (DDG group, n=22) or vaginal micronized progesterone, 800 mg/daily (VMP group, n=22), following endometrial preparation with transdermal estradiol. Patients who did not reach an endometrial thickness ≥ 7 mm or who developed a dominant follicle were excluded of the study. The main outcome was a viable ongoing pregnancy at 12 weeks of gestation assessed by ultrasound scans.
Main results and the role of chance: The median age of the patients was 34 years (DDG group) and 32 years (VMP group). The median endometrial thickness on the day of progesterone administration was 8.7 mm in DDG group and 9.2 mm in VMP group. In DDG group, one embryo transfer was performed in 50% (n=11) of patients, two embryos were transferred in 40.9% (n=7) of patients and three embryos were transferred in 9.1% (n=2) of patients. In VPM group, one embryo transfer was carried out in 36.4% (n=8) of patients and two embryos were transferred in 63.6% (n=14) of patients. Blastocyst stage transfer was performed in 81.8% (n=18) in DDG group and in 54.5% (n=12) in VMP group. A positive pregnancy test performed 14 days after FET was achieved in 45.5% (n=10) in both groups. Ongoing pregnancy at 12 weeks of gestation was observed in 36.4% (n=8) in DDG group and in 40.9% (n=9) in VMP group.
Limitations, reasons for caution: The investigation is ongoing and these are preliminary data. The number of patients is still small and the data must be confirmed with larger number of patients and analysis of the reproductive outcomes. At the present moment, no statistical analysis was performed and live-birth rate was not still assessed.
Wider implications of the findings: The use of oral dydrogesterone may be a more patient-friendly approach for endometrial preparation in FET, avoiding undesirable side effects and discomfort resulting from vaginal administration and providing similar reproductive outcomes.
Trial registration number: UTN:U1111-1247-1845 – Registro Brasileiro de Ensaios Clínicos

P-321 Intrauterine Granulocyte-Colony Stimulating Factor (IU G-CSF) to improve the suboptimal endometrium: Is it worth a try?

S. Ahuja[1], Z. El Mahdi[1], V. Shaikly[1], M. Ranieri[1], M. Taranissi[1]

[1]Assisted Reproduction and Gynaecology Centre, Assisted Conception, London, United Kingdom

Study question: Does intrauterine instillation of G-CSF improve the endometrial thickness in women with a sub-optimal endometrium (< 7 mm) going through an embryo transfer cycle?
Summary answer: In this retrospective study of 32 women, the endometrium improved to over 7 mm in 71.8 % of cases following IU G-CSF.
What is known already: The thin endometrium is a challenge to treat in assisted conception. Interventions to improve the endometrium include hysteroscopy to diagnose and treat intrauterine pathology, estrogen tablets/patches, sildenafil citrate orally/vaginally, vitamin E and l-arginine. Surrogacy is a last resort in women where the endometrial factor shows no improvement, using the above strategies. G-CSF is a cytokine reported to improve the thin endometrium and implantation. There are few published studies that have reported a positive impact of IU G-CSF on thin endometrium. This study adds to the existing literature.
Study design, size, duration: We report a retrospective study of 32 women who were identified to have a sub-optimal endometrium of < 7 mm during the treatment cycle over a period of 18 months. All the women were additionally on sildenafil citrate, aspirin and clexane to improve the endometrium. The women were counselled regarding the options of trying IU-GCSF versus cycle cancellation versus proceeding with the transfer if the endometrium was considered sub-optimal but suitable.
Participants/materials, setting, methods: Patients were selected for the intervention if their endometrium was below 7 mm in late stimulation (1-2 days before HCG trigger) or in the late estrogenic phase (day 10-12) in a medicated frozen embryo replacement cycle with sufficient serum estrogen levels. Following informed consent, 30MU in 1 ml G-CSF was instilled into the uterus. The endometrium was rechecked after 48 hours. A second dose was repeated if the endometrium was still sub-optimal.
Main results and the role of chance: Of the 32 patients, age 30-45 years, who received IU G-CSF, 20 women were having fresh and 12 women were having frozen embryo transfer cycles. 7 patients had a history of uterine pathology. The mean increase in endometrium was 1.23 mm with a range of 0 -3.7 mm. The endometrium increased to > 7 mm in 71.8 % of cases. The transfer was cancelled in 2 cases due to no change in the endometrium (6.6%). There were 8 pregnancies-3 have had livebirths, one of which was a set of twins, and one of them is about 30 weeks pregnant. 4 women had 1st trimester miscarriages. Only 1 woman complained of bone pain for about 1 week post IU G-CSF and her pregnancy sadly ended in a miscarriage.
The women in this study comprised of patients with complex fertility problems and previous treatment failures. Therefore, the positive and negative outcomes could have been due to other confounding actors such as embryo quality in the cycle rather than just the impact of the G-CSF on implantation.
Limitations, reasons for caution: The findings of this small retrospective study with no matched control, limit the conclusions that can be drawn. Furthermore, the women included in the study had complex fertility problems that could have contributed to the success/failure of the cycle independent of endometrium thickness.
Wider implications of the findings: Treating the sub-optimal endometrium is one of the most challenging situations in assisted conception. This study supports the evidence that IU G-CSF may have a positive impact. However, further research is required to establish its benefits and safety, as an option for patients before they consider alternative options like surrogacy.
Trial registration number: Not Applicable

P-322 Role of platelet rich plasma (PRP) in improving endometrial receptivity: A systematic review and meta-analysis.

H.S. Naseem[1], M. Lokman[1], F. Soxibova[2], L. Mohiyiddeen[1], Y. Abdallah[1]

[1]Manchester University NHS foundation Trust, Reproductive Medicine, Manchester, United Kingdom ;
[2]Medical University Sofia, Medical school, Bulgaria, Bulgaria

Study question: Does PRP improve outcome of assisted conception in women with unexplained recurrent implantation failure (RIF) or thin endometrium?
Summary answer: The use of PRP in women with unexplained RIF and thin endometrium has shown significant increase in chemical and clinical pregnancy rates through assisted conception.
What is known already: PRP is the concentrate obtained by centrifugation of the patient's own whole blood. Platelets have granules containing numerous proteins, several growth factors, and cytokines. In ex-vivo studies, activated PRP has been shown to promote the migration of endometrial stromal fibroblasts, endometrial mesenchymal stem cells and bone marrow-derived mesenchymal stem cells. This has formed the basis for in vivo use of autologous PRP to promote endometrial regeneration.It is also theorized that another mechanism of PRP action is via anti-inflammatory processes involved in receptivity of the endometrium and trophoblasts placentation. This would suggest that PRP could improve implantation and clinical outcomes.
Study design, size, duration: This is a systematic review of available literature including case series, cohort studies and randomised controlled trials (RCTs) on the role of PRP in improving endometrial receptivity in assisted conception. The databases were searched from inception to 2019. We then performed a meta-analysis, which comprised of four prospective RCTs comparing use of PRP intrauterine infusion or no treatment in women with unexplained RIF or thin endometrium. There are 330 patients included in the meta-analysis.
Participants/materials, setting, methods: This is a systematic review of available literature including case series, cohort studies and randomised controlled trials (RCTs) on the role of PRP in improving endometrial receptivity in assisted conception. The databases were searched from inception to 2019. We then performed a meta-analysis, which comprised of four prospective RCTs comparing use of PRP intrauterine infusion or no treatment in women with unexplained RIF or thin endometrium. There are 330 patients included in the meta-analysis.
Main results and the role of chance: The systematic review of current literature suggests a benefit of PRP intrauterine infusion in improving assisted conception outcomes. We performed a meta-analysis of available RCTs. Our inclusion criteria for the meta-analysis were: i) a prospective randomized controlled trial comparing PRP intrauterine infusion to controls in assisted conception, ii) participants were characterized as having thin endometrium or RIF, and iii) all patients had a negative hysteroscopy to rule out uterine factors.
134 relevant studies were identified. 92 were excluded as they did not meet the inclusion criteria. 30 studies were reviewed for full text and 4 were included in the meta-analysis.

The chemical and clinical pregnancy rate was reported in all 4 trials. The chemical pregnancy rate from pooled data showed a significant difference in favour of PRP treatment with odds ratio of 2.14 (OR: 2.14, 95% CI: 1.49 – 3.07; P<0.0001). Similarly, the clinical pregnancy rate from pooled data showed that there was a significant difference between the two groups (OR: 2.58, 95% CI: 1.75 - 3.78; P <0.0001).

Two trials reported on change to endometrial thickness and miscarriage rate. No significant difference was found for change of endometrial thickness or miscarriage rate, between the two groups.
Limitations, reasons for caution: The sample size in each RCT was relatively small and there was a lack of standardization in the PRP preparation. No trial reported measures to avoid performance or detection bias. These factors will impact the strength of evidence available.
Wider implications of the findings: These results support the evolving role for PRP in improving endometrial receptivity in clinical practice. Nevertheless, multi-centre RCTs with larger sample sizes are still needed to further evaluate the effectiveness of this intervention.
Trial registration number: Not Applicable

P-323 Prevalence of chronic endometritis varies more by diagnostic method than by reproductive history

M. Scarrone[1], S. Bordoli[1], V. Vanni[2], L. Quaranta[3], F. Pasi[4], C. Patruno[2], G. Campo[2], V. Canti[5], A. Quartucci[2], M. Castiglioni[4], M. Candiani[4], E. Papaleo[3], P. Rovere Querini[1], P. Giardina[3]

[1]Vita-Salute San Raffaele University, Internal and specialistic medicine, Milan, Italy ;
[2]Vita-Salute San Raffaele University, IVF Unit, Milano, Italy ;
[3]IRCCS San Raffaele Hospital, IVF Unit, Milan, Italy ;
[4]IRCCS San Raffaele Hospital, Obstetrics and Gynecology, Milan, Italy ;
[5]IRCCS San Raffaele Hospital, Internal and specialistic medicine, Milan, Italy

Study question: Does prevalence of chronic endometritis (CE) vary by reproductive history or by diagnostic method used?
Summary answer: CE is equally represented in patients affected by different types of reproductive failure. The different methods used for the diagnosis strongly influence its prevalence.
What is known already: CE is an asymptomatic inflammation of the endometrium mainly caused by bacteria. CE diagnosis is non-standardized and can be based on immunohistochemistry (IHC) for stromal endometrial CD138+ plasma cells or on endometrial cultures/molecular diagnosis for non-cultivable microorganisms. CE might be associated with impaired receptivity and reproductive failure, thus emerging as a potential factor in the pathogenesis of unexplained infertility, repeated implantation failure (RIF) and recurrent pregnancy loss (RPL). CE prevalence is inconsistent among studies, possibly due to different diagnostic methods and vaginal contamination of cultures.
Study design, size, duration: We performed a prospective study of patients referred to the hysteroscopic service of the San Raffaele Hospital (Milan, Italy) between May 2018 and December 2019 for diagnostic hysteroscopy and CE diagnosis as a second-level investigation for unexplained infertility (n=15), RIF (n=28) or RPL (n=44). We stratified CE prevalence by diagnostic method in addition to reproductive history.
Participants/materials, setting, methods: Patients underwent endometrial culture and concomitant vaginal and cervical cultures to account for possible contamination during sampling. A subgroup of n=43 samples were also subjected to immunohistochemistry for CD138+ plasmacytes. Diagnosis of CE was done in the presence of either a) positive endometrial culture/molecular bacterial DNA amplification or b) >=5 CD138+ plasmacytes in 20 high-power fields. Affected patients were treated with targeted antibiotic therapy and re-tested with endometrial culture to assess CE regression.
Main results and the role of chance: N=50 out of 87 cultures resulted positive (57.5%). In only 2 out of 50 cases, the same pathogen was isolated in both endometrial and vaginal cultures - with a concordance rate of 4% - suggesting that vaginal contamination is infrequent. IHC was positive in 12 out of 43 cases (27.9%). Concordant results between culture and IHC were observed in 25 cases, with a matching accuracy of 58,1%. Cases with contradictory results mostly showed positive bacterial cultures but negative immunohistochemical analysis (n=16 out of 18, 88.9%) while only n=2 out of 18 (11.1%) resulted positive at IHC but negative at culture. Of the n=22 affected patients who have completed treatment and control cultures at the time of data analysis, n=9 resulted negative (40.9%), n=6 (27.3%) tested positive for the same pathogen whereas n=7 (31.8%) tested positive for a different pathogen. We observed similar prevalence of CE among groups of patients stratified by reproductive history as defined by RIF (n=17/28, 60.7%), RPL (n=23/45, 52.1%) or unexplained infertility (n=10/15, 66.7%).
Limitations, reasons for caution: The currently small sample size is the main limitation of our study. Further prospective data collection and inclusion of pregnancy outcomes will be of interest.
Wider implications of the findings: Endometrial cultures are frequently positive in patients with reproductive failure. Our study shows that vaginal/cervical contamination has marginal role in this finding. However, only 58.1% of culture-positive patients show the presence of plasmacytes. Whether bacteria mediate endometrial dysfunction in the absence of plasmacytes-mediated inflammation needs further investigation.
Trial registration number: not applicable

P-324 The importance of timing in detection of asymptomatic CD138 diagnosed chronic endometritis

J. Odendaal[1,2], K. Fishwick[1], Q. Hussain[1], J. Thornton[1], K. Larsen[1], P. Vrljicak[1], E. Lucas[1], J.J. Brosens[1,2], S. Quenby[1,2]

[1]University of Warwick, Division of Biomedical Sciences, Coventry, United Kingdom ;
[2]University Hospitals Coventry and Warwickshire, Biomedical Research Unit, Coventry, United Kingdom

Study question: How does the expression of CD138 alter across the menstrual cycle?
Summary answer: CD138 is normally expressed, at mRNA and protein level, in glands and stroma during the proliferative phase but expression decreases markedly during the luteal phase.
What is known already: Asymptomatic chronic endometritis (CE), diagnosed by the presence of CD138-positive stromal cells on immunohistochemistry (IHC), has been associated with recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL). CE can be treated with antibiotics and has been linked with dysbiosis of the vaginal and endometrial microbiome. CD138 (syndecan 1) is a selectively expressed heparan sulphate proteoglycan and putative cell surface marker of plasma cells. However, CD138 expression occurs in non-immune cells and has been associated with proliferation, apoptosis and angiogenesis. The cycle-dependency and cellular distribution of CD138 expression in the endometrium has not yet been analysed comprehensively.
Study design, size, duration: Observational cohort study of (i) 27 proliferative and 29 luteal phase endometrial samples and 3 paired proliferative/luteal phase endometrial samples; (ii) interrogation of published single-cell RNA-seq data derived from 6 midluteal endometrial biopsies (GSE127918).
Participants/materials, setting, methods: Timed endometrial biopsies were obtained from local biobanks from control and recurrent reproductive failure patients. Endometrial *SDC1* (CD138) transcript levels were quantified by RT-qPCR and cellular distribution examined by single-molecule in-situ hybridization (smISH). Temporo-spatial expression of CD138 at protein level was examined by IHC followed by digital image analysis and deconvolution for quantification. Dual staining with Ki67 was performed to delineate CD138-positive proliferating cells. Single-cell transcriptomics data were interrogated to examine cell type-specific *SDC1* expression.
Main results and the role of chance: Whole tissue RT-qPCR demonstrated non-immune *SDC1* expression across the menstrual cycle with no significant difference in transcript levels across cycle phases. Analysis of midluteal scRNA-seq data showed enriched *SDC1* mRNA expression in endometrial epithelial cells, although transcripts were also detectable in stromal, immune and endothelial cell populations. The cellular distribution of *SDC1* transcripts was confirmed by smISH. IHC of proliferative endometrium demonstrated very high stromal CD138 expression (>200 CD138-positive stromal cells/10mm^2) in 26/27 samples. Expression was significantly reduced in luteal samples (Mann Whitney U-Test; P<0.005). This transition was also demonstrated across the paired proliferative/luteal samples. Dual IHC demonstrated a mean co-localisation of 30% of CD138-positive stromal cells with the proliferation marker Ki67 across the menstrual cycle, suggesting that CD138 is not merely marking proliferating cells. H-scoring of the glandular endometrium demonstrated a similar drop in expression from the proliferative to luteal phase (Mean H-Score 197.51 vs. 104.56; Student T-test; P<0.05).
Limitations, reasons for caution: The marked reduction in CD138 immunoreactivity upon transition from the proliferative to luteal phase was not reflected by a corresponding decrease in *SDC1* mRNA levels in whole biopsies. This discrepancy is likely due to post-transcriptional regulation of CD138 however, further work is required to elucidate this.
Wider implications of the findings: Detection of CD138-positive stromal cells is used increasingly for the diagnosis of CE. It is important that CD138 is assessed in the luteal phase to prevent over-diagnosis, that non-immune cell expression is acknowledged, and its clinical importance evaluated.
Trial registration number: Not applicable

P-325 Presence of focal adenomyosis lesion based on MRI is associated with infertility issues

L. Melka[1], M. Bourdon[1], J. Oliveira[1], J. Cormier[1], L. Marcellin[1], C. Maignien[1], C. Bordonne[2], A.E. Millischer[3], G. Plu-Bureau[1], P. Santulli[1], C. Chapron[1]

[1]Université de Paris- Faculté de Médecine- Paris- France, clinician, Paris, France ;
[2]Université de Paris- Faculté de Médecine- Paris- France, Radiology, Paris, France ;
[3]Centre de Radiologie Bachaumont- IMPC- Paris- France, radiology, paris, France

Study question: Is there an association between adenomyosis and infertility, according to the adenomyosis phenotype as diagnosed by magnetic resonance imaging (MRI)?
Summary answer: In the study population, the presence of focal adenomyosis was associated with primary infertility.
What is known already: Adenomyosis is characterized by the presence of endometrial glands and stroma deep within the myometrium. This disease exhibits various clinical presentations notably infertility, which prevalence and causes are still unknown. In addition, various forms have been described, including focal

(Foc-ADE) and diffuse adenomyosis (Dif-ADE), and several evidences suggest that those two forms could be considered as distinct entities, which could make the analysis of adenomyosis-related infertility even more difficult. As a matter of fact, the association between the distinct adenomyosis phenotypes (focal and/or diffuse forms) and infertility has not yet been investigated.

Study design, size, duration: This was a cross-sectional study using data prospectively collected in all non-pregnant patients aged between 18 and 42 years, surgically explored for benign gynecological conditions at our institution between May 2005 and May 2018. For each patient, a standardized questionnaire was completed during a face-to-face interview conducted by the surgeon during the month preceding surgery. Only women with uterine MRIs performed by a senior radiologist during the preoperative work-up were retained for this study.

Participants/materials, setting, methods: A uterine-MRI was performed in 496 women operated for a benign gynecological disease. Among them, 248 women did not present adenomyosis lesions and 248 women had a radiological diagnosis of adenomyosis. According to MRIfindings, women were diagnosed with Foc-ADE and/or Dif-ADE forms. Three groups were compared: the non-infertile women group (n=361), the 'primary infertility' group (n=84) and the 'secondary infertility' group (n=51). Univariate and multiple analysis were performed to determine primary and secondary infertility-associated factors.

Main results and the role of chance: The presence of focal adenomyosis was significantly associated with primary infertility. Diffuse adenomyosis was not found to be associated with infertility, whether at the primary or at the secondary stage. The distribution of other benign gynecological diseases, notably endometriosis or leiomyomas, was not significantly different between the groups. After a multinomial regression model including notably the women's age and the presence of endometriosis or leiomyoma, the presence of focal adenomyosis was identified as an independent associated factor of primary infertility (1.9; 95% CI: 1.1 - 3.3).

Limitations, reasons for caution: Inclusion of only surgical patients may constitute a possible selection bias. In addition, it cannot be excluded that other non–explored causes of infertility were present among the infertile women-group.

Wider implications of the findings: This study opens the door to future clinical and basic studies aiming at better characterizing focal adenomyosis and its infertility-related physiopathology

Trial registration number: NA

P-326 The correlation between gene expression in endometrial cells and the formation of pinopodes

I. Ilyin[1], O. Parnitskaya[2], N. Kazachkova[3], J. Gontar[3], Y. Gerevich[1], E. Kapustin[1], N. Buderatskaya[2], S. Lavrynenko[2], Y. Lakhno[3]

[1]Medical Centre IGR, ART department, Kyiv, Ukraine ;
[2]Medical Centre IGR, Embryology, Kyiv, Ukraine ;
[3]Medical Centre IGR, Diagnostic Laboratory, Kyiv, Ukraine

Study question: Do different patterns of gene expression affect morphology of the endometrial tissue in natural or stimulated cycles?

Summary answer: A specific pattern of gene expression contributes to the formation of a certain endometrial morphology, such as the appearance of pinopodes.

What is known already: Well known that recurrent implantation failure is tightly related with endometrial cells function. Endometrial structure and receptivity are result of gene expression in certain period of menstrual cycle. Many investigations indicated main genes of endometrial receptivity regulation in samples of infertile women. But it is equally important to understand how genes are expressed in the known morphology of endometrial tissue in fertile young women, such as oocyte donors in the natural menstrual cycle and during ovarian stimulation. Since there is an opinion that hormonal stimulation is ahead of the endometrium development in comparison with the natural cycle.

Study design, size, duration: The study was performed in the Medical Centre IGR from March 2018 to December 2019. It involved 42 endometrial samples obtained from 32 oocyte donors whose mean age was 27.9±2.8 (range 22-32 years). The parts of tissue sample were examined for detection of pinopodes, tissue structure and expression of following genes generally upregulated during receptive phase (URG) *PAEP,GPX3, TAGLN,EDNRB, CLU, HABP2,LMOD1, IMPA2, GAS, FXYD2* and such downregulated genes (DRG) as *HLA-DOB, CTNNA2, CAPN6, MAP2K6, NDRG2,SORD*.

Participants/materials, setting, methods: The whole endometrial tissue sample was received by pipelle biopsy from 32 women on the mid-luteal phase of the natural menstrual cycle or on the day of oocyte pick up. Each biopsy was separated into three parts:one was fixed in 10% formaldehyde for histopathological analysis, the other piece was fixed in 2.5% glutaraldehyde for scanning electron microscopy (SEM) and the third part in PBS was taken for gene expression detection using qPCR analysis.

Main results and the role of chance: Of 42 samples 33 were obtained from women in the natural menstrual cycle and 9 samples were taken after oocyte retrieval, moreover 9 women also had been sampled in the natural cycle prior to ovarian stimulation start. The expression of URG in edometrium cells was higher in stimulated cycles (n=7), but statistically it wasn't significantly different (SSD) (p>0,05), the expression pattern of the DRG was the same in natural and stimulated cycles. Histopathological analysis of each sample also was similar and showed type of tissue structure appropriate early secretion.

According to the results of SEM, all samples were divided into 3 groups: (A) no signs of pinopodes formation (n=31), (B) increased number of ciliary cells without pinopodes formation (n=9) and (C) the presence of pinopodes in the samples (n=2). Group A had pattern with very low expression of URG and higher DRG. Group B samples demonstrated higher expression of URG and high expression of some DRG. And group C samples showed high level expression of URG and low expression of DRG similar to expression profile during receptive phase; that showed a SSD calculated by Chi-square on levels of gene expression relative to groups A and B (p<0,001).

Limitations, reasons for caution: Tracking changes in endometrial morphology using SEM and specific genes expression is a useful tool in predicting of embryo implantation. But these indicators are very variable depending on the individual characteristics of the patient as well as the selected drugs and dosages correctness in the cycles of ovarian stimulation.

Wider implications of the findings: A study of the morphology and gene expression of endometrial samples obtained from donor is the basis for assessing the status of the endometrium in patients with infertility, which allows us to more accurately understand the problem of implantation failure and adjust treatment in the right direction

Trial registration number: Not applicable

P-327 Bowel endometriosis-related infertility: assisted reproductive technology (ART) outcomes are favorable in women with no prior history of surgery for endometriosis

C. Maignien[1], P. Santulli[1], M. Bourdon[1], L. Marcellin[1], K. Pocate-Cheriet[2], J. Gonnot[1], V. Blanchet[1], C. Chapron[1]

[1]Assistance Publique-Hôpitaux de Paris AP-HP- Centre Hospitalier Universitaire CHU Cochin, Department of Gynaecology Obstetrics II and Reproductive Medicine, Paris, France ;
[2]Assistance Publique-Hôpitaux de Paris AP-HP- Centre Hospitalier Universitaire CHU Cochin, Department of Reproductive Biology, Paris, France

Study question: To evaluate the ART cumulative live birth rates (CLBR) in a cohort of bowel endometriosis (OSIS) patients with no prior history of surgery for OSIS

Summary answer: ART CLBR are excellent (67.3%) in bowel OSIS patients with no prior history of surgery. No bowel complications occurred during ovarian stimulation or pregnancy

What is known already: Bowel OSIS-related infertility is a therapeutic challenge, with 2 main treatment modalities: surgery and ART, each with different risks of complications. Numerous reports have been published about ART pregnancy rates after bowel surgery, but data are currently lacking on ART outcomes in bowel OSIS affected-women who reverted to ART first. Moreover, the determinant factors of pregnancy chances in this specific population remain unclear

Study design, size, duration: Observational cohort study on 101 consecutive bowel OSIS patients without a prior history of surgery for OSIS, who underwent IVF-ICSI treatment at Cochin-Port-Royal Hospital between January 2013 and December 2018. Diagnosis of OSIS was based on published imaging criteria using transvaginal sonography and magnetic resonance imaging. Patients with a prior history of surgery for OSIS were excluded

Participants/materials, setting, methods: The main outcome measure was CLBR. Miscarriage rates, perinatal outcomes and bowel complications were also studied. We compared the characteristics of women who became pregnant and those who did not, using univariable and multivariable analysis, to identify determinant factors of fertility outcome

Main results and the role of chance: One hundred and one bowel OSIS patients underwent 176 ART cycles. The mean number of deep infiltrating endometriosis lesions per patient was 3 ± 0.9, with a mean number of bowel lesions

of 1.3 ± 0.6. Seventy-three percent of the patients had associated endometriomas, and 88.1% had associated adenomyosis. Overall, the cumulative live birth rate was 67.3%. No bowel complication occurred during ART cycles or pregnancies. Regarding perinatal outcomes, cesarean sections were performed in 38.2% of the pregnancies; there were 7.4% preterm births (< 37 WG) and 10.3% low birthweight (< 2500 g). Using multivariate analysis, AMH levels <2 ng/mL ($p < 0.001$) and antral follicle count < 10 ($p = 0.006$) were the only factors associated with negative ART outcomes

Limitations, reasons for caution: The diagnosis of OSIS was based on imaging rather than surgery. This limitation is however inherent to the design of the studies on OSIS patients reverting to ART first

Wider implications of the findings: CLBR are excellent in bowel endometriosis patients undergoing ART first, with low risks of complications. Low ovarian reserve seems to be the main prognostic factor of ART CLBR. These data urge the clinician to carefully weight the pros and cons before systematically referring infertile bowel OSIS patients to radical surgery

Trial registration number: NA

P-328 Deep endometriosis with digestive involvement : Comparison of the surgical strategy and ATR

R. Goldstein[1], S. Bendifallah[2], S. Touleimat[3], H. Roman[3], F. Krief[4], M. Ballester[5], E. Darai[1,2], 2. Bendifallah.S([1]

[1]Department of Gynaecology and Obstetrics, Tenon University Hospital, Assistance Publique des Hôpitaux de Paris ;
[2]INSERM UMRS 707, Epidemiology, Information Systems ;
[3]Expert Center in the Diagnosis and Multidisciplinary Management of Endometriosis, Department of Gynecology and Obstetrics, Rouen University Hospital, Rouen ;
[4]Hôpital Jean verdier, Department of gynecology-obstetricics, Bondy ;
[5]Hôpital Diaconesse, Department of gynecology-obstetricics, Paris ;
[6]Hôpitaux de Saint Maurice, Department of gynecology-obstetricics, Saint Maurice

Study question: To compare the fertility results of two therapeutic strategies for colorectal endometriosis associated-infertility :surgery followed by spontaneous conception+ / - IVF (In vitro fertilization) versus IVF in first intention.

Summary answer: For women with colorectal endometriosis associated-infertility, first-line surgery seems to be a good option, especially for patients with good prognostic factors.

What is known already: surgery for deep endometriosis improved fertility. Regarding endometriosis with digestive involvement, data in the literature are scarce. Their is no consensus for the management of those patients. . Regarding endometriosis with digestive involvement, data in the literature are scarce. Their is no consensus for the management of those patients.

Study design, size, duration: Retrospective cohort study conducted in hospital Tenon in Paris and in the University hospital of Rouen, two referral centers in endometriosis. Patients: 159 patients were inclued between January 2010 and December 2016

Participants/materials, setting, methods: The patients were matched after using a propensity score (PSM). The propensity score was developed using a logistic regression model, with the following covariates : age, anti-mullerian hormone (AMH) and the presence of adenomyosis.113 patients were included in the surgery first group and 46 patients in the IVF first group. Patients follow-up was done for a period of at least 24 months after surgery or the first IVF cycle.

Main results and the role of chance: After PMS, The pregnancy rate was 76% (86/113) in the primary surgery group (including 33.7% (29/86) after spontaneous conception and 66.3% (57/86) after IVF), versus 30.4% (14/46) in the IVF first group. The live birth rate was 50.4% (57/113) in the primary surgery group (including 45.6% (26/57) after spontaneous conception and 54.4% (31/57) after IVF), versus 23.9% (11/46) in the IVF first group. Overall the 182 cycles performed, the cumulative rates of live birth after the first IVF cycle in the surgery first group compared to the IVF first group were 29.6% versus 15.2%, for the second cycle 44.2% versus 28.6% and for the third cycle 69.6% versus 40.5%.For patients oriented towards spontaneous conception attempt after surgery, the pregnancy rate was 69% (29/42) and the live birth rate 61.9% (26/42), with a median time of 6 months [IQR: 5-11 months] for pregnancy. Pregnancy and live birth rates were significantly higher in the surgery first group compared to the IVF first group, especially for the subgroup of patients with good prognostic factors (AMH> 2 ng / mL, age <35 years and no adenomyosis).

Limitations, reasons for caution: retrospective study, selection bias (Two referral centers of endometriosis surgery), difference between the 2 groups (3 for 1 pairing after PSM)

Wider implications of the findings: Pregnancy and live birth rates were significantly higher in the surgery first group compared to the IVF first group, especially for the subgroup of patients with good prognostic factors (AMH> 2 ng / mL, age <35 years and no adenomyosis).
This study highlights a good live birth rate after surgery for the spontaneous pregnancy, with a median time of 6 months.

Trial registration number: not applicable

POSTER VIEWING SESSION
ETHICS AND LAW

P-329 Analysis of the outcomes chosen by european patients for their surplus embryos, taking into account all possible options available in Spain

M. Lopez-Teijon[1], V. Walker[1], C. Castello[1], B. Marques[1], S. Novo[1], F. Garcia[1], A. Garcia-Faura[1]

[1]Institut Marques, Reproductive Medicine Service, Barcelona, Spain

Study question: What outcome do patients choose for their surplus frozen embryos?

Summary answer: Only 13,3% of patients who have surplus embryos make an active choice about their final outcome. 86,7% don't respond, or keep the embryos for themselves.

What is known already: Fertility clinics continue to accumulate frozen embryos. Deciding what to do with surplus embryos can be difficult for patients and can create significant emotional conflict.
It's not that patients have an irresponsible attitude towards their embryos; rather it appears to be the long-reaching implications that their decision might have. Patients don't want to give the embryos to other women for fear their children might one day meet their siblings; they don't want to destroy them because it makes them feel bad, and they don't want to donate them to research because they are frightened of what might be done to them.

Study design, size, duration: In accordance with Spanish law, patients sign a consent form confirming their desired outcome for their surplus embryos. All options are open to them, and every 2 years after freezing the patients receive a letter asking them to reconfirm or alter their previous decision.
We assessed the informed consents from 7607 patients doing IVF (own eggs and donor eggs) between 2012 and 2016 with surplus frozen embryos. Patient follow-on replies up to 2018 were analysed.

Participants/materials, setting, methods: Patient consent forms and replies were analysed and the chosen outcome for the embryos noted. We compared the replies given by patients from different european countries and also the decisions made by patients regarding embryos made from their own gametes or from donor gametes.

Main results and the role of chance:

1. B. 41% wished to maintain the embryos for their own use. 90% of these patients had completed their family, they didn't want any more children but they still preferred to keep their surplus embryos.
   C. 2% destroyed the embryos
   D. 3% gave them away to others
   E. 3% donated them to research

There were no significant differences in the results according to the biological origin of the embryos. There were though notable differences between the options chosen by patients from countries in close geographical proximity, which highlights the idiosyncrasies of each culture.

Ireland: A 45%, B 45%, C 1,5%, D 7%, E 1,5%
UK: A 63%, B 29%, C 3%, D 3%, E 2%.
Germany: A 36%, B 51%, C 6,5%, D 6%, E 0,5%.

France: A 61%, B 25%, C 7%, D 4%, E 3%.
Spain: A 56%, B 33%, C 2%, D 3%, E 6%.

Limitations, reasons for caution: The study did not differentiate between replies from couples or single patients. Therefore in the majority of cases it is a joint decision made by the couple, and maybe if just one partner was asked to decide the results would have been different.
Wider implications of the findings: When couples finish their reproductive project their surplus embryos create a problem they do not know how to resolve.

If they don't answer the letters sent to them, embryos are considered abandoned. Spanish law allows fertility clinics to choose the outcome for these embryos, that can include entering the embryo-adoption program.
Trial registration number: not applicable

P-330 Import/export of cryopreserved gametes and embryos between European centers: what should be done, which forms, which protocols, which responsibilities: an Italian experience

C. Garello[1,15], B. Dal Canto[2,15], E. Baldi[3,15], M. Cervi[4,15], S. Dabizzi[5,15], D. Farace[6,15], D. Giacchetta[7,15], M. Menegazzo[8,15], M.G. Minasi[9,15], M. Oneta[10,15], L. Rienzi[11,15], G. Taliani[12,15], D. Paoli[13,15], L. De Santis[14,15], On behalf of SIERR[15]

[1]LIVET s.r.l., IVF Unit, Torino, Italy ;
[2]Zucchi Clinical Institutes, Biogenesi Reproductive Medicine Center, Monza, Italy ;
[3]University of Florence, Experimental and Clinical Medicine, Florence, Italy ;
[4]Healthcare "Friuli Occidentale", IVF Unit, Sacile PN, Italy ;
[5]Careggi Hospital, Unit of Andrology- Female Endocrinology and Gender Incongruence, Florence, Italy ;
[6]University of Rome Tor Vergata, Department of System Medicine, Rome, Italy ;
[7]San Paolo Hospital, Laboratory of Seminology and male gametes cryopreservation, Milan, Italy ;
[8]University Hospital, Andrology and Reproductive Medicine, Padova, Italy ;
[9]European Hospital, Center for Reproductive Medicine, Rome, Italy ;
[10]Sacco Clinical Sciences Institute, Reproductive Unit Department of Obstetric and Gynaecology, Milan, Italy ;
[11]Clinica Valle Giulia, Genera Centres for Reproductive Medicine, Roma, Italy ;
[12]Sapienza of Rome University, Umberto I Hospital, Rome, Italy ;
[13]Sapienza University of Rome, Laboratory of Seminology-Sperm Bank "Loredana Gandini", Rome, Italy ;
[14]San Raffaele Scientific Institute, Ob/Gyn IVF UNIT, Milan, Italy ;
[15]SIERR, Italian Society of Embryology- Reproduction and Research, Italy, Italy

Study question: is standardization of National and International procedures and forms for cryopreserved samples transfer sufficient to meet quality, traceability and safety requirements?
Summary answer: Indications on shipping between tissue institutions and legal responsibilities are important points and a working protocol with shared transport forms has been defined.
What is known already: In Italy, the transport of cryopreserved biological material is dealt exhaustively by several Legislative Decree (No. 191/2007;16/2010 and D Lgs 10 October 2012) which transpose European Directives. Given the nature of their application, the transport of reproductive cells has peculiar quality and safety requirements that must be applied univocally, minimizing the chance of error. In order to standardize the transborder shipping procedure to meet the quality, traceability and safety criteria for cells and tissues, it is appropriate to establish a unified process using the same tools, forms and communication channels.
Study design, size, duration: The Italian Society of Embryology, Reproduction and Research (SIERR) has defined a working protocol with shared forms in order to distribute them nationally and internationally. This is necessary in light of the increasingly widespread movement of biological samples between the various countries.
Participants/materials, setting, methods: A working group has been created by SIERR. This "FOCUS Group" was constituted by representatives from Italian ART centres and Sperm Banks who worked together to define joint procedural steps and create specific forms to support movement of cryopreserved samples.
Main results and the role of chance: The FOCUS Group identified the critical steps in the communication procedures between centres and created the related forms: patient authorisation, request from the recipient centre, critical checks carried out by both sending and recipient centres, start of samples transfer, collection, transport and taking responsibility of the biological material, acknowledgment of samples arrival, acknowledgement of any adverse event occurred.
Limitations, reasons for caution: SIERR will evaluate the validity of these modules through several pilot SIERR affiliated ART centres in order to evaluate the concrete application and usefulness of the forms.
Wider implications of the findings: Transfer of cryopreserved samples between centers implies effective and appropriate on time communication in order to establish the approaches of transport itself, in terms of ethical, legal and practical management. It is increasingly essential to share a homogeneous procedure that guarantees the management of cryopreserved gametes and embryos.
Trial registration number: not applicable

P-331 PGT-A in any Egg Donation

A. Anastasi[1], F. Capodanno[1], S. Rossi[1], A. Gallinelli[1]

[1]AUSL di Ferrara - Ospedale del Delta di Lagosanto, Centro di Fisiopatologia della Riproduzione Umana, Ferrara, Italy

Study question: Why Pre implantation Genetic Test for Aneuploid diseases (PGT-A) should be routinely performed in Egg Donation (ED)?
Summary answer: Only euploid embryos should be routinely transferred after ED. Following a stressful and expensive ED, embryo transfer with euploid-blastocyst should be strongly recommended.
What is known already: Data from EIM for ESHRE, suggest that 39 Countries and 1.279 Institutions perform ART, with a total of 56.516 ED. No informations about ED following embryo-transfer with euploid-embryos are available. Even if egg-donors are young, aneuploidy increases in women older than 26. A slightly increased prevalence of embryo aneuploidy is reported in young patients, with >40% aneuploidy in women 23 years and less. Aneuploidy can not be excluded in young egg donors.
IVF Centers discourage PGT-A following ED, without any scientifically reason. We speculate that PGT-A in ED is not perfomed with the questionable aim to guarantee fresh and following frozen-embryo-transfer.
Study design, size, duration: To perform PGT in all ED, offering to the patients the higher chances to transfer an euploid embryo. It would be advisable to set a duration of 2 years in order to evaluate the cost-effective of this approach.
Participants/materials, setting, methods: The are 39 Countries and 1.279 Institutions offering ART services, 56.516 treatment cycles with egg donation (ED) where the study could be conducted.
Main results and the role of chance: The main goal is to establish a clinical setting that it offer to the couples submitted to ED the highest chances to transfer one euploid embryo, optimizing the costs of the procedure. The second objective is the cost-effective evaluation of the policy we suggest: cost-effectiveness analysis is an hot topic in the debate about clinical application of PGT-A, but we assume that the high cost of new techniques for the genetic screening such as the Next Generation Sequencing (NGS), could be surely covered by high number of couples seeking PGT after ED. Recent studies suggest a positive cost-effective ratio only in women older than 35 years old. However, no data are available regarding cost-effectiveness of PGT-A in younger patients or in those submitted to ED. In our view, PGT-A could allow to optimize the cost of ED, allowing to the couples to pay for euploid embryos, and not for the aneuploid ones. The question we should make us in term of costs is the following: how much any aneuploid embryo cost respect to any euploid one?

The unique way to support couples hopeful having euploid gametes is PGT-A, especially in ED where financial and psychological investment of infertile women are consistent.
Limitations, reasons for caution: PGT-A following ED could flow in the indiscriminate employment of this technique, with a consequent uncontrolled business.

PGT-A following ED is difficult to adopt because of financial, social and organizational reasons.
Wider implications of the findings: Optimization of financial and psychological investment of couples submitted to ED.
To get more data about aneuplody frequency in egg donors.
Trial registration number: not applicable

P-332 Ethical concern regarding the assisted reproduction techniques: a qualitative interview study with professionals, patients, donors and general population

J. Guijarro[1], R. Nuñez-Calonge[2]

[1]Hospital Virgen de la Luz- Cuenca, Obstetricia y Ginecología, Cuenca, Spain ; [2]Duncan, Ethics and reproduction, Madrid, Spain

Study question: Is there a different ethical consideration regarding the assisted reproduction techniques of professionals compared to patients, donors and general population?
Summary answer: There is a different moral consideration towards the problems derived from assisted reproduction techniques, being higher in physicians and laboratory personnel and lower in donors.
What is known already: Assisted reproduction techniques (ART) have opened numerous ethical questions different from religious or legal approaches, involving not only the professionals who perform them but also patients and donors. Additionally, many ethical challenges generated by new scientific developments in this field generate widely differing opinions.

To date, there are no studies comparing the concerns of the different moral agents involved in these processes and whether the ethical issues raised are the same in all these groups.
Study design, size, duration: The plan was set-up as an electronic qualitative survey study and took place between September and December 2018.
Participants/materials, setting, methods: Data were collected from 123 voluntarily participating assisted reproductive centres (including clinicians, embryologists, nurses and administrative staff) and 110 non-professionals (oocyte and sperm donors, patients and general population). Participants filled out an anonymous questionnaire asking for their socio-demographic characteristics and 100 questions based on the main ethical problems. After a depuration process by means of Factorial Analysis a definitive questionnaire with 41 items was obtained.
Main results and the role of chance: Based on the interviews, we articulated four main topics describing participants views regarding the ethical aspects of assisted reproduction: solidarity, morality, religiosity and confidence. Comparing the average of scores in the four issues according to the professional relationship with assisted reproduction it was concluded that the professional / non-professional dichotomy is the one that occupies more extreme values: the professionals display a greater preoccupation by the morality, a greater confidence and a more restrictive attitude, without influence by religiosity. Conversely, non-professionals present a more solidary and mistrustful profile, with greater disregard for moral issues. Within the group of professionals, doctors have a greater moral concern than the rest and a less supportive attitude.

People without a direct relationship with reproduction have a more supportive and distrustful profile, similar to the patients, but clearly present a greater indifference for moral problems. Donors are the group that presents less concern for ethical or moral aspects and greater confidence in medical decisions.
Limitations, reasons for caution: The non-existence of religious differences in the surveyed population may have led to a bias in some answers.

A possible effect of social desirability in the answers by the patients should be considered.
Wider implications of the findings: The differences in systems of values demonstrate the need to have a thorough discussion on the specific meaning of the ethics terms.

The personal profile can be useful in clinical practice when dealing with the patient in an individualized manner.
Trial registration number: Not applicable

P-333 Artificial womb technology and the ethics of prenatal medicine

S. Segers[1]

[1]Bioethics Institute Ghent - Ghent University, Department of Philosophy and Moral Sciences, Gent, Belgium

Study question: How would artificial womb technology affect how obligations towards the future child and obligations towards the expecting mother are balanced against each other?
Summary answer: Artificial womb technology could facilitate prenatal interventions and may exacerbate potential conflicts between directive treatment recommendations and the pregnant woman's autonomous decision to the contrary.
What is known already: Current developments in artificial womb technology have rekindled the expectation that human ectogenesis may come within reach. As yet, the ethics debate has largely focused on the link with abortion, how this technology may provide an alternative for surrogacy or uterus transplantation and how it could remedy gender inequity and enhance reproductive autonomy. So far, the morally relevant connection between ectogenesis and the fetus as a beneficiary of treatment is largely overlooked. Ectogenesis could provide easier access to the fetus and thus contribute to the allocation of a patient status to the fetus.
Study design, size, duration: A literature study of the scientific literature was performed to inventory the state-of-the-art of artificial womb technology and a literature study of the respective bioethical literature was performed to inventory the main ethical arguments. Next, ethical problems in prenatal medicine were conjoined with normative argumentation to arrive at substantiated ethical judgements with regards to the ethics of ectogenesis and decision making in prenatal medicine.
Participants/materials, setting, methods: Literature study, conceptual analysis, normative analysis.
Main results and the role of chance: Suggestions have been made that ectogenesis could make it easier for clinicians to fix fetal defects by allowing to treat the fetus outside the woman's body, inside the artificial womb. Combined with social expectations about maternal responsibilities, this may put pressure on pregnant women to undergo prenatal interventions for the benefit of the future child. However, despite an expected better fetal accessibility, concerns for the pregnant woman's wellbeing still remain in case of surgical removal and physical translocation of the fetus to the artificial uterus. While a pregnant woman may have obligations of beneficence towards the fetus, she may nevertheless refuse interventions, not only because of the value of autonomy, but also that of bodily integrity. An argument can be made that a focus on fetal beneficence should not disregard potential cases of autonomy violation as connected with a person's life goals and personal interests (like the woman's wish to carry the fetus in the womb to birth). As such, ectogenesis aided fetal treatment could induce higher tensions between a clinician's obligations towards the pregnant woman and the future child. It is defended here that ectogenesis would not circumvent the key ethical concerns that come with in utero fetal treatment.
Limitations, reasons for caution: Human ectogenesis is still hypothetical, especially the variant where the offspring's development happens from conception to birth completely outside a woman's uterus. This paper focuses on partial ectogenesis, meaning that 'ectogenesis' will generally refer to the transfer of a partially developed fetus to an artificial womb for further development.
Wider implications of the findings: The availability of ectogenesis aided fetal treatment could open the door for routine directive counselling for fetal benefit. With the advent of artificial womb technology, it is timely to inquire whether actual clinical codes of practice are sufficiently fine-grained to provide ethical guidance to the future practice of prenatal medicine.
Trial registration number: BOF19/24J/066

P-334 The ethical implications of the framing of egg donation on Belgian fertility clinic websites

L. Jacxsens[1], C. Coveny[2], L. Culley[3], T. Goethals[1], C. Herbrand[4], S. Lafuente[5], V. Pavone[6], G. Pennings[7], V. Provoost[7], C. Weis[3], N. Hudson[3]

[1]Ghent University, Bioethics Institute Ghent, Ghent, Belgium ; [2]Loughborough University, Department of Social Sciences, Loughborough, United Kingdom ; [3]De Montfort University, Centre for Reproduction Research, Leichester, United Kingdom ; [4]De Montfort University, Centre for Reproduction Research, Leicester, United Kingdom ; [5]Goethe-Universität Frankfurt am Main, Faculty of Social Sciences, Frankfurt am Main, Germany ; [6]Consejo Superior de Investigaciones Cientificas, Institute of Public Goods and Policies, Madrid, Spain ; [7]Ghent University, Bio-ethics, Ghent, Belgium

Study question: How is egg donation framed on the websites of fertility clinics in Belgium and what are the ethical implications of this framing?
Summary answer: Donors were presented as mothers under 37; recipients as heterosexual couples with a medical need. Overall, egg donation was framed as a medical act.

What is known already: How information is presented or framed can significantly influence the interpretation and decisions of the reader. Therefore, the framing of egg donation in information for prospective donors is ethically important. In the literature, the most important ethical debates concerning egg donation deal with the topics of commercial egg donation, postmenopausal pregnancies and anonymity. Studies (mostly conducted in the United States) have shown that the framing of gamete donation is gendered and that altruism seems to play an important role. This is the first frame analysis of the Belgian context.
Study design, size, duration: This study is part of the ESRC-funded 'EDNA' project that aims to explore the social, political, economic and moral configuration of egg donation in the United Kingdom, Belgium and Spain. In this study we only focused on Belgium.
Participants/materials, setting, methods: We analysed the websites of all 18 Belgian fertility clinics that perform egg donation. Frame analysis and content analysis were used. The websites of clinics in Flanders and Wallonia were analysed separately.
Main results and the role of chance: On Belgian clinic websites egg donation is presented as a service that suffers from scarcity in donors. Images found on clinic websites were mainly in the style of commercial advertisement images, with the exception of a small number of websites where 'scientific' images were used. Overall, the donor was presented as someone who is (preferably) a mother, younger than 37 years, and highly motivated, typically because she donates to or for (in the case of cross donation) family or friends. The recipients were framed as heterosexual couples who are in need of eggs due to medical reasons. In general, egg donation was framed as a medical act. Information relating to risks mainly dealt with physical aspects whilst psychological risks were less frequently mentioned. Minor risks (such as temporary physical discomfort) and more serious risks (e.g. ovarian hyperstimulation syndrome) received equal coverage. All websites where compensation was mentioned made it clear that this is not a payment and only one website mentioned the amount of compensation. On the Flemish websites expressions of clinic preference for anonymous donation were present whereas this was not found on the Walloon websites.
Limitations, reasons for caution: Clinic websites are the first step in a process of information provision that continues in the clinic. The current analysis is limited to one country. In a further phase, these data will be combined with data from the two other countries (UK and Spain) involved in the EDNA-project
Wider implications of the findings: The presentation of donor or recipient profiles is ethically important since people who do not relate to these profiles might feel excluded, thus dismissing egg donation as an appropriate practice. The framing of risks has ethical implications for the donors' informed consent.
Trial registration number: ES/N010604/1

P-335 Relational autonomy in postmortem sperm retrieval and donation

H. Mertes[1]

[1]*Bioethics Institute Ghent BIG Ghent University, Department of Philosphy and Moral Sciences, Gent, Belgium*

Study question: Should relational autonomy be the standard instead of personal autonomy in the case of postmortem sperm retrieval and donation?
Summary answer: As genetic material 'belongs' to several family members and the disadvantages of the procedure fall disproportionately on family members, a relational approach should be pursued.
What is known already: Recently, a study was published pleading to include the option of postmortem sperm donation in organ donor programmes in order to increase the availability of donor sperm. This is technically feasible and if one relies on the analogy with organ donation, one could argue that it is the deceased's right to decide how his tissues are used after passing away. At the same time, several legal cases have been brought forward in which family members request control over reproductive material of a deceased person, some of which were successful.
Study design, size, duration: This is a normative analysis in which (a) different arguments from the literature and from legal cases are inventoried, compared and scrutinized; (b) the analogy with posthumous organ donation is dismantled, looking for semantic and structural (in)consistencies and (c) an exploration is made of how the concept of relational autonomy might be operationalized in this case and whether this would lead to better or worse outcomes for the different stakeholders.
Participants/materials, setting, methods: Literature research; normative analysis
The method that is used to bring empirical data (as found in literature research) and normative ethics together is the 'Wide Reflective Equilibrium', the most commonly used method in bioethics.
Main results and the role of chance: Personal autonomy is a very highly valued principle within biomedical ethics, yet it has been criticized for ignoring the fact that people are essentially relational, interconnected beings, that people's identities are shaped by those with whom they interact and that one person's decisions have implications for others. Feminist scholars have therefore introduced the concept of relational autonomy, which emphasizes the importance of input from the web of people a patient is connected with in medical decision making. While one should be very wary of this concept being abused to sideline autonomous choice, posthumous sperm donation may be one case in which a relational approach can be well defended. In first instance, this is because genetic material is concerned, over which people can be said to have shared 'ownership', rather than individual ownership (and thus decisional authority). Secondly, the benefits for the donor are dubious, whereas the donor's action can cause significant distress and grievance in his family. Also, the benefits for the recipients are of a different order than in the case of organ donation. Sharing the decisional authority between family members therefore seems appropriate.
Limitations, reasons for caution: What does not follow from this reasoning is that family members would have decisional authority over posthumous sperm retrieval and use in the absence of advance directives by the deceased. In this case the family would seek a benefit at the expense of the deceased's autonomy, instead of avoiding harm.
Wider implications of the findings: Although personal autonomy is and should be a central concept in reproductive ethics, incorporating relational autonomy can lead to new perspectives on how to deal with reproductive decision making.
Trial registration number: N/A

POSTER VIEWING SESSION
IMPLANTATION AND EARLY PREGNANCY

P-336 Anterior gradient protein 3 and S100P levels in the reproductive tract mucosa may play an important role in human fertility

N. Tempest[1,2], **E. Batchelor**[2], **J. Drury**[2], **D. Hapangama**[1,2]

[1]*Liverpool Women's Hospital, Obstetrics and Gynaecology, Liverpool, United Kingdom ;* [2]*University of Liverpool, Department of Women`s and Children`s Health- Institute of Translational MedicineLiverpool, Liverpool, United Kingdom*

Study question: Do women with implantation problems have altered levels of two endometrial calcium binding proteins (Anterior grade protein 3 (AGR3) and S100P) when compared with women of proven fertility?
Summary answer: Significantly higher AGR3 and S100P immunostaining was observed in cilia of luminal epithelium (LE) of women with implantation problems compared with women of proven fertility.
What is known already: Calcium signalling pathways are of vital importance to embryo implantation. Calcium-binding proteins such as S100P, Anterior gradient protein 2 (AGR2) and Anterior gradient protein 3 (AGR3) participate in these pathways. AGR2 and S100P have previously been shown to be involved in endometriosis and endometrial cancer and S100P demonstrates highest endometrial levels during the window of implantation (WOI). Endometrial expression and the functional relevance of AGR3 is unknown, but it may play an important role in the regulation of ciliary beat frequency, thus in the LE of the endometrium may play an important functional role in embryo transfer and fertility.
Study design, size, duration: This observational study examined luteinising hormone timed human endometrial samples taken in the WOI from 53 women (10 recurrent miscarriage (RM), 13 recurrent implantation failure (RIF), 30 control). The women underwent gynaecological procedures at a UK tertiary referral centre. The women did not have any known endometrial pathology and had not been on hormone treatment for 3 months prior to the procedure.

Participants/materials, setting, methods: Immunohistochemistry was used to confirm the presence of and immuno-staining patterns of AGR3 and S100P in human endometrium during the WOI. Immuno-reactivity was analysed with a quick score that quantified immunostaining in the different endometrial areas at the cellular level.

Main results and the role of chance: In total, 53 patients (10 RM, 13 RIF, 30 control) with an average age of 39 years were included in the study. The women in the RM group had an average of 4 previous miscarriages, while women in the RIF group had an average of 8 previous failed embryo transfers and the control group on average had 2 previous children.

The epithelial cell sub-region and subcellular location of S100P immunoreactivity was different in the healthy fertile controls when compared with the RM and RIF samples. Nuclear S100P was seen in control samples whereas RM/RIF samples showed no evidence of nuclear S100P immunoreactivity in the WOI. Cilia of the LE of the RM/RIF samples demonstrated significantly higher ($p<0.01$) S100P immuno staining when compared with the control samples.

Similar differences were observed in AGR3 immunoreactivity of the ciliated LE cells, where significantly higher immune-scores were demonstrated by RM/RIF samples ($p<0.01$).

This observed higher immunoreactivity for 2 calcium binding proteins in the LE cilia of women with a history of reproductive failure suggests their inaccurate subcellular location associated pathophysiology for these conditions. The nuclear localisation of S100P may allow transcriptional regulatory function that is necessary for implantation of a viable pregnancy.

Limitations, reasons for caution: This study employs only immunohistochemistry and although we have confirmed gene expression with qPCR no functional studies have been conducted to confirm a role of the 2 calcium binding proteins in the implantation process.

Wider implications of the findings: We describe the presence of epithelial AGR3 in the human endometrium and demonstrate differential AGR3 and S100P in the WOI in women with a history of reproductive failure. These proteins may have a functional role in fertility. Further work is thus warranted to assess their utility as a diagnostic/therapeutic targets.

Trial registration number: NA

P-337 Placental disorders of pregnancy and In-Vitro Fertilization – does placental histological examination explain the excess risk?

H. Ganer Herman[1], L. Tamayev[2], O. Feldstein[2], M. Bustan[2], Z. Rachmiel[2], L. Schreiber[3], A. Raziel[1], B. Jacob[2], M. Kovo[2]

[1]Edith Wolfson Medical Center, In Vitro Fertilization, Holon, Israel ; [2]Edith Wolfson Medical Center, Obstetrics and Gynecology, Holon, Israel ; [3]Edith Wolfson Medical Center, Pathology, Holon, Israel

Study question: Assessment of clinical characteristics of in vitro fertilization (IVF) pregnancies complicated by fetal growth retardation (FGR) and preeclampsia (PE), and the correlation to histopathological placental findings.

Summary answer: Neonatal outcome is relatively favorable in placental diseases in IVF pregnancies. Placental villitis is more common in IVF pregnancies, pointing to an additive immunological etiology.

What is known already: Assisted reproduction is correlated with an increased risk for FGR and PE, independent of the higher incidence of risk factors among IVF patients. FGR and PE, termed "the placental diseases of pregnancy", share a common pathogenesis of ischemia, with a higher rate of vascular mal-perfusion placental lesions. Villitis of unknown etiology is an additional placental finding in FGR, assumed to reflect an immunological pathogenesis.

Study design, size, duration: This was a retrospective cohort of 1114 deliveries during an 11-year period.

Participants/materials, setting, methods: The study was performed in a tertiary hospital. The cohort included deliveries with a diagnosis of FGR and/or PE, with a singleton live birth at 22 weeks of gestation or more, whose placentas were sent to histological examination. We compared obstetric, neonatal and placental characteristics between IVF conceived pregnancies and controls.

Main results and the role of chance: A total of 1114 deliveries with a diagnosis of placental related pregnancy complications were included - 105 conceived with IVF (IVF group) and 1009 non-IVF conceived (control group). Patients in the IVF group were older, and of lesser parity and gravidity. The rate of diabetes mellitus and chronic hypertension was significantly higher among IVF patients, and the rate of smokers significantly lower. Patients in the IVF group delivered at a significantly earlier gestational age, 36.6 ± 3.2 vs. 37.3 ± 2.9 weeks, p=0.02. A trend for a higher rate of cesarean delivery was noted in the IVF group, 69.5% vs. 60.7%, p=0.07, while birth weight was non-significant between the groups. A significantly higher rate of neonates in the control group experienced an adverse neonatal outcome. No difference in placental mal-perfusion lesions was demonstrated between the groups. However, a significantly higher rate of villitis of unknown etiology (VUE) was demonstrated in the IVF group, 16.1% vs. 8.3%, p=0.007. A sub-analysis of stimulated cycles, frozen embryo transfer cycles and controls, demonstrated a significantly higher rate of VUE among stimulated cycles as compared to controls (21.0% vs. 8.3%, p=0.006), with no significance between stimulated and frozen cycles, or frozen cycles and controls.

Limitations, reasons for caution: Despite a departmental protocol necessitating placental examination in complicated pregnancies, the study cohort probably represents more severe cases of FGR and PE, for which placental examination was performed. In addition, certain data was unavailable and underpowered for sub-analysis, such as IVF cycle characteristics (stimulated/frozen).

Wider implications of the findings: PE and FGR are common among IVF pregnancies, although neonatal outcome is more favorable in this group. Placental examination demonstrated a higher rate of VUE in IVF pregnancies, pointing to an additional immunological pathogenesis, thus contributing to our knowledge regarding the excess risk for placental disorders in IVF pregnancies.

Trial registration number: N/A

P-338 Proteomic characterization of endometrium in IVF-ET patients with repeated implantation failure

C. Wang[1], H.Y. Zhang[1], Z.J. Cheng[1], Y. Feng[1], W.J. Zhou[1], M.Y. Jiang[1], Y. Zhou[1], X.Y. Fei[1]

[1]Hangzhou Women's Hospital Hangzhou Maternity and Child Health Care Hospital, Reproductive Medicine Center, Hangzhou, China

Study question: Repeated implantation failure (RIF) seriously affects the clinical pregnancy rate of in vitro fertilization-embryo transfer (IVF-ET) treatment, while the pathogenesis of RIF was still unknown.

Summary answer: We highlighted antithrombin-III and corticosteroid-binding globulin as RIF-related proteins by screening the RIF patients' endometrium proteins.

What is known already: Assisted reproductive technology has developed for more than 40 years, but there is no significant breakthrough in pregnancy rate. RIF is a difficult problem in IVF-ET treatment. The uterine environment, especially the endometrial receptivity during the implantation window, has a great influence on the pregnancy of such patients.

Study design, size, duration: In this study, we collected the endometrium of IVF-ET patients in our hospital during 2016 to 2019. According to the pregnancy outcomes, we finally gained 40 RIF cases. According to the patients' age, infertile factors, and visiting time, we matched our RIF cases with 40 pregnant cases.

Participants/materials, setting, methods: All the female patients were aged under 40. We performed iTRAQ-2D LC-MS/MS between RIF and pregnant group. Clinical data were analyzed using paired t test.

Main results and the role of chance: Fifty-five higher expressed proteins (> 1.50 times, $P < 0.05$) and 27 lower expressed proteins (< 0.67 times, $P < 0.05$) were found significant changed by iTRAQ-2D LC-MS/MS in RIF patients. Furthermore, the most different categories: negative regulation of hydrolase activity, blood particles, and enzyme inhibitor activity were found in the biological process, cell composition and molecular function through GO analysis with hypergeometric test. We also found that the differential proteins were mostly in the immune system, and mRNA translation through KEGG analysis, while most of them were enriched in the primary immunodeficiency. At last, we discovered that these RIF-related proteins were clustered mostly in the post-translational modification, protein turnover, chaperones; transduction, ribosome structure and biogenesis; and function unknown using the EggNOG database. The most repeated proteins were antithrombin-III and corticosteroid-binding globulin, in the different categories we found by GO analysis, which related to hypercoagulable state and lower progesterone level, respectively. However, we found higher level of D-Dimer and lower level of progesterone in RIF patients with no significant differences ($P > 0.05$).

Limitations, reasons for caution: Most of the proteins that screened by iTRAQ-2D LC-MS/MS were lack of commercialized ELISA Kit or antibody, and

we could only compare paired RIF and pregnant cases' clinical data to reveal the hypercoagulable state and lower progesterone level of RIP patients. More cases of clinical data were needed.
Wider implications of the findings: Our results might give a new sight into the implantation-related molecular, moreover, also provides a new experimental basis to understand the pathogenesis of RIF.
Trial registration number: Project supported by the Medicine and Health Science Technology project of Zhejiang province,China(2017KY550), the Natural Science Foundation of Zhejiang province,China (LQ18H040009).

P-339 Circ-CCNB1 inhibits trophoblast cells proliferation and inasion by sponging miR-223 to regulate SIAH1 during embryo implantation

D. Wang[1], L. Xie[1], M. Jin[1], Y. Zhou[1], A. Liu[1]

[1]School of Medicine- Zhejiang University, Reproductive endocrinology, Hangzhou, China

Study question: Whether circRNAs regulate trophoblasts function and the tentative molecular mechanisms in aberrant implantation environment remains unknown.
Summary answer: circ-CCNB1 regulates trophoblast cell proliferation and invasion through sponging miR-223 to increase SIAH1 expression and inhibit CCNB1 nuclear translocation.
What is known already: Ectopic pregnancy (EP) has been recognized as a model to investigate the signaling and pathways regulated by trophoblast cells during embryo implantation. Circular RNAs (circRNAs) have been reported to exert important regulatory effects on trophoblasts function and embryo development.
Study design, size, duration: To study the tentative molecular mechanisms in the occurrence of EP, five trophoblast cells samples from EP and five trophoblast cells samples from normal pregenancy were used to analyze the differential expression of circRNAs by high-throughput sequencing. To determine the effect of circ-CCNB1 on trophoblast cells function, JEG-3 and HTR-8 cells were transfected with overexpression of circ-CCNB1 vectors or empty vectors for 24 h or 48 h *in vitro*.
Participants/materials, setting, methods: Through high-throughput sequencing, we found the decreased expression of (circRNA CyclinB1) circ-CCNB1 in the patients with EP. Subsequently, overexpression of circ-CCNB1 vectors, CCK-8, transwell, immunofluorescence, quantitative PCR, western blot, RNA pull-down and dual-luciferase reporter assays were used to investigate the effect of circ-CCNB1 on the function of trophoblast cells.
Main results and the role of chance: *In vitro* study demonstrated that overexpression of circ-Ccnb1 slightly inhibited JEG-3 cell proliferation and significantly inhibited HTR-8 cell proliferation after 24h and 48h treatment. Moreover, transfection of circ-Ccnb1 overexpression vectors lead to a significant decrease of cell invasion in JEG-3 and HTR-8 cells. Furthermore, miR-233 was predicted and demonstrated to interact with circ-Ccnb1 in trophoblast cells. We found miR-233 mimics treatment decreased the expression levels of circ-Ccnb1, whereas miR-233 inhibitor significantly enhanced the expression levels of circ-Ccnb1 in trophoblast cells. Meanwhile, miR-233 inhibitor treatment suppressed cell proliferation and invasion in HTR-8 cells. Interestingly, we found miR-233 dramatically increased cyclin related genes CCNB1, Thymidine kinase (TK1) Krüppel like factor-4 (KLF4) and nuclear factor kappa B (NF-κB) expression levels, while significantly decreased the expression levels of E3 ubiquitin ligase, seven in absentia homolog-1 (SIAH1) and F-box and WD-40 domain protein 7 (FBXW7), and apoptotic factor caspase-3. SIAH1 was testified to be directly targeted by miR-233, which could be activated by circ-Ccnb1 treatment in trophoblast cells. In addition, we also found circ-Ccnb1 increased CCNB1 expression levels and suppressed CCNB1 nuclear translocation in HTR-8 cells.
Limitations, reasons for caution: The investigation of the molecular mechanism of circ-CCNB1 regulating trophoblast cells function was limited in trophoblast cells *in vitro*. It's necessary to testify this mechanism in the EP animal models in future.
Wider implications of the findings: These results demonstrated an important molecular mechanism of circ-CCNB1 regulating trophoblast cell proliferation and invasion through sponging miR-223 to increase SIAH1 and inhibit CCNB1 nuclear translocation, which might provide a novel pathophysiological characteristic during embryo implantation.
Trial registration number: 20180192

P-340 Can AUTOLOGOUS PLATELET RICH PLASMA treatment improve pregnancy outcome in ivf/et cases : a case control study done at Teerthanker Mahaveer Medical College & RC

P. Singh[1]

[1]TMMC&RC, OBS & GYNAE / IVF, MORADABAD, India

Study question: Can AUTOLOGOUS PLATELET RICH PLASMA treatment improve pregnancy outcome in ivf/et cases ?
Summary answer: the use of autologous PRP holds promise in the treatment of women with suboptimal ET and vascularity for embryo transfer.
What is known already: Poor endometrium is a cause of treatment faliure in majority of cases of assisted reproduction .refractory endometrium is seen in many cases which does not respond to various treatments e.g. vaginal silnafil , esrtogen,androgens,low dose aspirin etc.to overcome this refractory endometrium PRP treatment was found effective .
Study design, size, duration: CASE CONTROL STUDY
18 MONTHS
168 PATIENTS WERE INCLUDED IN STUDY.
Participants/materials, setting, methods: Intrauterine instillation of autologous PRP was done in 168 women,101 did not received aprp treatment due to various reasons,taken as controls between 22 and 45 years, over 18 months, with suboptimal endometrial growth, and patients with repeated cycle cancellations, in addition to vaginal sildenafil ,Estradiol valerate etc . Embryo transfer was performed when the endometrium reached an optimal pattern in terms of thickness, appearance, and vascularity.
Main results and the role of chance: The mean pre-PRP endometrial thickness (ET) was 3.5 mm which significantly increased to 7.18 mm post-PRP. There was a significant increase in vascularity, seen by the number of vascular signals seen on Power Doppler, reaching the zones 3 of the endometrium. The positive beta Human Chorionic Gonadotropin (hCG) rate was 70.93% and the clinical pregnancy rate was 42.31%. A total of 47 delivered healthy babies ,11 women are in the second trimester, 12 are in the first trimester with a healthy intrauterine pregnancy, one patient had an ectopic gestation, 22 had missed abortions.
Limitations, reasons for caution: Small sample size
Wider implications of the findings: Patients with refractory endometrium ,especially in younger age groups seems to be benifitted by this technique.
Trial registration number: NA

P-341 Impact of uterine contractility on pregnancy success rate during in-vitro fertilisation: assessment by quantitative ultrasound imaging

C. Rees[1], C. Blank[1], N. Kuijsters[2], Y. Huang[2], F. Sammali[2], H. Van Vliet[1], P. De Sutter[3], M. Mischi[2], B.C. Schoot[1]

[1]Catharina Hospital Eindhoven, Obstetrics and Gynaecology, Eindhoven, The Netherlands ;
[2]Eindhoven University of Technology, Signal Processing Systems, Eindhoven, The Netherlands ;
[3]Ghent University Hospital, Reproductive Medicine, Ghent, Belgium

Study question: Can assessment of uterine activity in patients with successful in-vitro fertilisation (IVF) versus patients with unsuccessful IVF treatment be used to predict success of embryo implantation?
Summary answer: Measuring uterine contractility prior to embryo implantation in IVF patients is able to predict chance of pregnancy with 93.8% accuracy.
What is known already: During the menstrual cycle, the natural contraction pattern of the uterus is affected by changes in hormones. Abnormal uterine contraction activity is known to influence the outcome of fertility treatment. Recently, an automated measurement tool to characterise these movements has been developed, but has not yet been widely applied to patients receiving fertility treatment.
Study design, size, duration: A single-centre prospective study included 16 IVF/ICSI patients between January 2017 and July 2018. Participants underwent three quantitative 2D-transvaginal ultrasound measurements prior during three phases of IVF treatment: follicle stimulation (FS), one hour before embryo transfer (ET1), and five to seven days after ET (ET5-7).
Participants/materials, setting, methods: A dedicated speckle tracking algorithm was implemented to extract frequency and amplitude-related features

of uterine motion in relation to ongoing implantation at 6 weeks up to 11 weeks pregnancy. Machine learning and random forest models were used to create a prediction model based on uterine motion characteristics prior to ET in order to suggest a subsequent chance of pregnancy.

Main results and the role of chance: Overall, frequency and amplitude of uterine significantly decreased during the phases of the IVF/ICSI treatment showing FS as the most active phase followed by ET1 and ET5-7. Women with successful implantation/pregnancy showed significantly higher uterine contraction frequency compared to those without in all phases. Conversely, uterine contraction amplitude was significantly lower (p = 0.037) in women at ET5-7 in case of with ongoing implantation/pregnancy. Analysing uterine contraction frequency and coordination prior to ET was able to predict chance of pregnancy after ET with a sensitivity of 85.7%, specificity of 100% and an accuracy of 93.8%.

Limitations, reasons for caution: The small sample size limits statistical power of this study. Research should be continued with larger patient cohorts to confirm the results. Additionally, not all uterine motion characteristics were taken into account, in future research the full characterisation of the uterine activity during IVF treatment should be completed.

Wider implications of the findings: This study is a first step to objectively and non-invasively assess uterine contraction characteristics during IVF cycles, and introduces a new method to predict chance of IVF success. In future, it may boost IVF success rates by for example introducing specific uterine motion modulators prior to ET.

Trial registration number: NL5035

P-342 Oral Dydrogesterone vs. Micronized Vaginal Progesterone(MVP) Gel for Luteal Phase Support (LPS) in Frozen-Thawed Single Blastocyst Transfer in Good Prognosis Patients

G. Özer[1], K.B. Yuksel[1], O.S. Yucel Cicek[2], S. Kahraman[1]

[1]Istanbul Şişli Memorial Hospital, Memorial Hospital- ART and Reproductive Genetics Center- Istanbul., Istanbul, Turkey ;
[2]Kocaeli University School of Medicine, Department of Gynecology and Obstetrics, Kocaeli, Turkey

Study question: Is oral dydrogesterone an effective option for luteal phase support in modified natural cycle frozen -thawed embryo transfers (mNC-FET) when compared to MVP gel?

Summary answer: In mNC-FET cycles, oral dydrogesterone provides an effective option compared to MVP gel with similar ongoing pregnancy rates and with fewer intolerable side effects.

What is known already: Administration of progesterone for LPS could be oral, intramuscular, vaginal and subcutaneous with each route having various bioavailability, side effects and tolerability profiles. The optimal form of progesterone supplementation has not been determined from available data. Since bioavailability of oral micronized progesterone is <10 % and is related to drowsiness, flushing, and nausea, it is not commonly used for LPS. However, dydrogesterone, which is an optical isomer of progesterone, has better bioavailability and progestogenic activity and causes endometrial transformation at a dose of 10–20 fold lower than that of oral micronized progesterone.

Study design, size, duration: This was a randomized, single center, parallel controlled trial conducted at Istanbul Memorial Hospital IVF and Reproductive Genetics Centre, Istanbul, Turkey between January and August 2019. A total of 134 women, aged below 38, were assigned randomly in a ratio of 1:1, based on a computer-generated list to receive oral dydrogesterone (n=67) or MVP (n=67) for LPS in mNC-FET cycle.

Participants/materials, setting, methods: The exclusion criteria were a history of ≥2 unsuccessful cycles and early pregnancy losses, uterine malformation, severe endometriosis, azoospermia and women with endocrine or metabolic disorders

The primary outcome was ongoing pregnancy rate (OPR). Secondary outcomes were clinical pregnancy and miscarriage rates, patients' satisfaction and tolerability of oral and vaginal progesterone. A questionnaire was developed to compare side effects and satisfaction profiles. Data was collected through telephone interviews on the day of pregnancy test.

Main results and the role of chance: There was no significant difference in demographic features such as female age, body mass index, AMH levels and fresh cycle characteristics including the number of retrieved oocytes, mature oocytes, ICSI-fertilized oocytes, the number of vitrified blastocysts and endometrial thickness on the day of recombinant-hCG between two groups (p>0.05).

When we compared the results of mNC-FET cycles, OPR was 68.7% in MVP gel and 71.6% in dydrogesterone group, respectively (p=0.706). Biochemical - clinical pregnancy rates and biochemical - clinical miscarriage rates were also comparable between two groups (p=0.382, p=0.547, p=1.0, p=1.0 respectively).

A significantly higher patient tolerability score was present in the dydrogesterone arm (4,13±0,97 vs 3,43±1,17, p=0.001).

Vaginal discharge (76,9% vs. 12,3%, p=0.001), vaginal irritation (44,6% vs. 1,6%, p=0.001) and interference with coitus (50% vs. 1,6%, p=0.001) were significantly higher in MVP gel compared to dydrogesterone group. On the other hand, mastalgia (36,9 % vs. 4,6%, p= 0.001), headache (13,8% vs. 0%, p=0.003), dizziness (9,4% vs. 0%, p=0.013), flatulence (32,8% vs. 12,3%, p=0.005) and somnolence (38,5% vs. 1,5%, p=0.001) were also significantly higher in the dydrogesterone group compared to MVP gel.

Limitations, reasons for caution: In order to avoid any possible selection bias, which can result from compromised clinical features, only good prognosis patients with top and good blastocysts were included in the study.

Wider implications of the findings: When choosing the form of progesterone supplementation, effectiveness of treatment, as well as side effects, patients' preference and convenience should be considered. Dydrogesterone, as an alternative oral preparation for LPS, constitutes an effective option, especially for patients suffering from side effects of vaginal progesterone.

Trial registration number: NCT04124913

P-343 Embryos versus endometrium: who is to blame for the impaired implantation rate in morbidly obese women?

I. Antonova[1], T. Milachich[1], I. Bochev[1], M. Yunakova[2], M. Magunska[2], A. Shterev[2]

[1]SAGBAL "DR. SHTEREV" EOOD, Embryology, Sofia, Bulgaria ;
[2]SAGBAL "DR. Shterev" EOOD, Ivf, Sofia, Bulgaria

Study question: What is the major reason for the reduced implantation and clinical pregnancy rates in cases with obese women who have undergone ART treatment?

Summary answer: Our results suggest that the endometrial receptiveness is adversely affected by higher proportions of the body fat and leads to difficulties in pregnancy achievement.

What is known already: Obesity has become one of the greatest public health challenges of the last few decades. Its prevalence has tripled in many countries of the WHO European Region since the 1980s, and the numbers of those affected continue to rise at an alarming rate. Overweight female patients undergoing fertility treatment face many complications. Obesity drastically increases gonadotrophin consumption and is linked with lower embryo implantation and higher miscarriage rate as well. Elevated body mass index (BMI) is associated with complicated obstetric anamnesis such as risk of pre-eclampsia and gestational diabetes.

Study design, size, duration: This is retrospective analysis from hospital database for 5-year period. This study includes 3599 fresh cycles using own gametes and additional group of 343 patients with donor oocytes as a control group. For the purpose of the investigation, all patients were divided into 4 groups according the international BMI WHO classification: underweight (BMI<18.49) group A (n=336); normal range (BMI 18.5-24.9), group B (n=2291); overweight (BMI 25-29.9), group C (n=639) overweight and D obese (BMI>30) (n=333).

Participants/materials, setting, methods: In order to establish possible impact of excess weight on oocyte and embryo quality, we compared average number of the retrieved oocytes, fertilization rate, embryo quality at day 3 and blastocyst rate between the groups. For the examination of the linkage between obesity and endometrial receptivity, we measured main outcomes such as pregnancy and implantation rates and compared the results with a control donor-oocyte recipient group.

Chi-squared analysis was used to compare results.

Main results and the role of chance: All four groups were homogeneous according the average number of the retrieved oocytes (5.97 vs 6.11 vs 5.99 vs 6.25; p: NS) and regarding the fertilization rate: 73.5% for group A, 74.4% Group B, 73.6% group C and 73.3% for group D respectively.

At cleavage stage we measured the embryo quality in groups and they showed similar proportions of good, fair and bad embryos. Day 5 blastocyst rate was slightly higher in normal BMI group B 45%), but not significant referred to other groups (A - 42% vs C - 42% vs D - 40%).

After clinical pregnancy rates (CPR) measurement groups A and B appeared comparable results (34.6% vs 35.7%). In overweight group (C) CPR was lower, but not significantly (31.5%); (P = 0.0895). Statistical difference was found in obese group D where CPR dropped to 28.9% (P = 0.0392). In the control group of 343 patients using ovum donation the results were even more demonstrative where CPR was 56% for underweight women (n=33); 52.8% for normal range (n=208); 50% in overweight (n=70) and only 17.4% in the obese group (n=32) .

Limitations, reasons for caution: This is observational retrospective study and it is limited by the subgroups size. More detailed studies are required to achieve a deeper understanding and to shed light into the finer aspects of this complicated implantation process.

Wider implications of the findings: Our survey shows that the endometrium receptiveness is the one that is negatively influenced by excess fat cells. This could be a good predictive model for implantation capability as there is a clear cut-off range in BMI values. Weight-loss concealing before fertility treatment could be beneficial for the successful outcome.

Trial registration number: not applicable

P-344 Endometrial steroid metabolism (intracrinology) in relation to pregnancy outcome in women undergoing IVF/ICSI

L. Brentjens[1], J. Den Hartog[1], B. Delvoux[1], M. Häkkinen[2], J. Koskivuori[2], P. Koskimies[2], S. Auriola[2], N. Van Hoogenhuijze[3], H. Torrance[3], F. Broekmans[3], SCRaTCH-2 study group(4), R. Van Golde[1], A. Romano[1]

[1]Maastricht University Medical Centre - GROW Maastricht University, Obstetrics & Gynaecology, Maastricht, The Netherlands ;
[2]University of Eastern Finland, School of Pharmacy, Kuopio, Finland ;
[3]Universitair Medisch Centrum Utrecht, Divisie Vrouw en Baby - Gynaecologie & Voortplantingsgeneeskunde, Utrecht, The Netherlands ;
[4]Amsterdam Medisch Centrum, F. Mol, Jeroen Bosch Ziekenhuis

Study question: Does the endometrial steroid profile (estrogens, progestogens, androgens and corticosteroids) differ between receptive and non-receptive endometrium?

Summary answer: The levels of steroids were similar in the endometrium of women who did and did not achieve a clinical pregnancy through IVF/ICSI.

What is known already: Implantation failure of high-quality embryos is a main concern in IVF/ICSI treatment. We previously reported that in IVF/ICSI patients, blood sex-steroid concentrations do not reflect their corresponding concentrations in endometrial tissue. This is in line with the concept that blood steroids (and precursors) are locally converted to bioactive metabolites and vice versa, leading to a specific intra-tissue steroid milieu. The endometrium regulates local steroid concentrations by expressing steroid-metabolising enzymes, or 'intracrinology'. Studies indicate that alterations in intracrinology might modulate endometrial receptivity. We hypothesize that the endometrial steroid profile during the window of implantation differs between pregnant and non-pregnant IVF/ICSI patients.

Study design, size, duration: Case-control study of 40 patients that were recruited in the SCRaTCH study (NTR 5342), a randomised trial exploring whether 'endometrial scratching' in patients with a previous IVF/ICSI cycle failure affects pregnancy outcome in a subsequent IVF/ICSI cycle. For the present investigation, 20 endometrial biopsies from women who became clinically pregnant after fresh embryo transfer were compared with 20 endometrial biopsies of women that did not conceive after fresh embryo transfer.

Participants/materials, setting, methods: Endometrial biopsies and serum were obtained at LH+ 5-8 days (urinary test) in a natural cycle. In their next cycle, subjects underwent IVF/ICSI with fresh embryo transfer. Cases (negative pregnancy test, n=20) and controls (clinically pregnant, n=20) were selected and matched for primary vs. secondary infertility, embryo quality and age. Steroid concentration was determined in endometrial 'scratched' tissue homogenates and serum (liquid chromatography mass spectrometry). Statistics was computed with Pearson Correlation and unpaired Student's *t*-test.

Main results and the role of chance: Steroids were measured in 40 endometrial biopsies and 16 serum samples. Median estrone level was 0.23 pmol/g in tissue and 0.29 pmol/mL in serum. Median estradiol level was 0.37 pmol/g in tissue and 0.45 pmol/mL in serum. Tissue progesterone (P4) median was 51.32pmol/g; P4 in serum was above quantification limit (33.3pmol/mL). With regard to androgens, median androstenedione level was 2.02pmol/g in tissue and 6.06pmol/mL in serum, whereas the median testosterone level was 0.34pmol/g and 0.86pmol/mL in tissue and serum, respectively.

Among corticosteroids, median 11-deoxycortisol level was 0.32 pmol/g in tissue and 0.64 pmol/mL in serum. Androsterone in tissue was below quantification limit (<3.26pmol/mL) and median serum level was 0.60 pmol/mL.

No statistically significant differences were found in steroid **tissue** concentrations between cases and controls. Mean **serum** 11-deoxycortisol levels were higher in women who conceived compared with those who did not (1.38±1.24 vs. 0.45±0.23 pmol/mL, p=0.04). The opposite was seen for androsterone, whose mean serum levels were lower in women who conceived compared with women who did not (0.47±0.23 vs 0.72±0.10 pmol/mL, p=0.02).

Tissue and blood concentrations were not correlated for estrogens, indicative of local steroid metabolism of these steroids, but they were significantly correlated for most progestogens, androgens and corticosteroids.

Limitations, reasons for caution: Even though cases and controls were matched for several important variables, it cannot be excluded that other maternal factors or embryo aneuploidy could have led to implantation failure, as there is heterogeneity in IVF/ICSI treatment indication in the (small) group of not-pregnant women.

Wider implications of the findings: Further clarification of the complex interplay between endometrial steroidogenesis, the tissue availability of steroidogenic enzymes, and that of the steroid hormone receptors in women with multiple failed IVF/ICSI cycles is necessary to unravel a possible role in the implantation process.

Trial registration number: NTR5342

P-345 Impaired myeloid-derived suppressor cells are associated with recurrent implantation failure

H. Jiang[1], Y. Cao[1], K. Wang[1], K. Bi[1], Z. Lu[1], P. Guo[1], C. Li[1]

[1]the First Affiliated Hospital of Anhui Medical University, Obstetrics and Gynecology, Hefei, China

Study question: Studies have reported that myeloid-derived suppressor cells (MDSCs) contribute to maintain pregnancy. The aim of this case–control study was to test whether there is a dysregulation of peripheral MDSCs in recurrent implantation failure (RIF).

Summary answer: This study indicated that the dysregulation of MDSCs might impaired maternal-fetal immune balance thus result in RIF.

What is known already: Recurrent implantation failure (RIF) is diagnosed when women experienced 3 or more frozen or fresh cycles with being transferred high-quality embryos and failed to obtain a clinical pregnancy. Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous cell group with attributes in myeloid origin, immature state, and immunosuppressive function.

Study design, size, duration: In our study, We collected 26 RIF cases and 30 controls 2019. Then we completed the experiments in two months.

Participants/materials, setting, methods: 26 RIF patients and 30 controls were recruited. Flow cytometry was applied to characterize polymorphonuclear (PMN)-MDSCs, monocytic-MDSCs (M-MDSCs), effector T cells (Teffs) and regulatory T cells (Tregs) in blood. ELISA was used to define MDSCs correlative cytokines and chemokines in serum from all patients.

Main results and the role of chance: Compared with controls, RIF patients showed significant reductions of blood PMN-MDSCs, M-MDSCs, Tregs and NO production by PMN-MDSCs, whereas the expression of ζ chain on $CD4^+$T cell receptor (TCR) and $CD8^+$TCR displayed a remarkable upregulation in RIF patients. Moreover, RIF patients presented a lower concentrations of serum chemokine (C-C motif) ligand (CCL) 5 and transforming growth factor (TGF)-β than those from controls. Furthermore, the level of TCR ζ chain on $CD4^+$ and $CD8^+$Teffs was negatively correlated not only with the percentage of PMN-MDSCs, but also with the amount of NO produced by PMN-MDSCs. The frequency of PMN-MDSCs had positive correlations with the concentration of CCL5 and TGF-β.

Limitations, reasons for caution: Consistent with previous observations, results showed Treg failure in patients with RIF. However, the proportion of Treg in this study is independent of the proportion of MDSC. This may be because hormones can also induce Tregs.

Wider implications of the findings: our study discovers the role of MDSCs and their related mediators in RIF. Therefore, targeting these cells could provide new treatment methods in the future.

Trial registration number: 1808085QH273

P-346 Validation of an in vitro model to study the interactions between endometrial cells, uterine natural killer cells and embryonic signals during human implantation.

A. Almansa[1], C.S. King[2], P. Brighton[2], G. Tiscornia[1], G. Hartshorne[2], J. Muter[2], M. Barragan[1], J. Brosens[2], R. Vassena[1]

[1]Clinica Eugin, Eugin, Barcelona, Spain ;
[2]Warwick Medical School, University of Warwick University Hospital Coventry and Warwickshire, Covnetry, United Kingdom

Study question: What is the effect of human embryo conditioned media obtained from blastocysts of different quality on uterine natural killer (uNK) cell activity during implantation?
Summary answer: Conditioned medium from blastocysts that failed to give rise to biochemical pregnancy diminished uterine NK mediated clearance of senescent endometrial cells *in vitro*.
What is known already: Embryo implantation is a regulated process involving synchronous communication between the embryo, endometrium and immune system. Recent studies demonstrate the emergence of acute cellular senescence during decidualization. Senescent cells irreversibly exit the cell cycle and remain biologically active, but may be detrimental to endometrial function if left unchecked. uNKs infiltrate the endometrium and are present in high numbers during implantation where they neutralize senescence by selectively targeting and eliminate the senescent decidual cell population. Here we validate a model for the uNK clearance of senescence, and apply it to investigate interactions between different endometrial cell types involved in this process.
Study design, size, duration: Basic research study. We co-cultured decidualized human endometrial stromal cells (EnSCs) from 5 women with human uNK cells in the presence embryo-conditioned media (ECM) obtained from cultured human embryos that were transferred as part of IVF treatment. Transferred embryos were grouped depending on whether they resulted in a positive biochemical pregnancy or not. Non-conditioned culture media was used as control. The effect of conditioned media on uNK cell clearance was measured.
Participants/materials, setting, methods: EnSCs were cultured from 5 different endometrial biopsies. uNK were isolated by MACS targeting CD56, and purity and viability assessed by flow cytometry. Stromal cells were decidualised for 6 days with 8-bromo-cAMP and medroxyprogesterone acetate, before uNKs were added. After 2 days of co-culture, levels of senescence associated-β-Galactosidase (SA-β-Gal) and senescent-specific genes *DIO2* and *CLU* were used as direct indicators of uNK clearance. Effects of ECM were assessed by inclusion at day 6.
Main results and the role of chance: uNK cells isolated from endometrial biopsies were round, granulated and stained positively for CD56. Characterization by flow cytometry revealed high purity (83.5%) and viability (87.4%). As expected, we observed the emergence of decidual senescence in EnSCs by increased SA-β-Gal, and expression of *DIO2* and *CLU*. The addition of uNK cells in co-cultures effectively eliminated cellular senescence by significantly reducing SA-β-Gal (p=0.038), *DIO2* (p=0.046) and *CLU* (p=0.021). Markers for non-senescent, mature decidual cells (*SCARA5* and *CXCL14*) were unaffected. This demonstrates the specific uNK-mediated targeting and elimination of senescent decidual cells. The effects of embryo-derived signals on uNK function were assessed by inclusion of ECM at day 6. Exposure to non-conditioned media, or media from embryos that resulted in positive biochemical pregnancies had no effect on uNK cell mediated senescence clearance. However, on evaluation of SA-β-Gal we detected a significant loss of clearance activity when the system was cultured with ECM from embryos that failed to establish pregnancies (p=0.015). We propose that embryonic signals from poor quality embryos inhibit uNK function. This demonstrates our model can detect uNK cell biological activity in response to embryo-derived stimuli and could be useful as an improved model to help understand human implantation
Limitations, reasons for caution: This study was conducted with biopsies from a limited number of patients; higher numbers of samples will need to be characterized to confirm our findings. Furthermore, our model lacks the endometrial epithelial component involved in apposition of the embryo to the endometrium.
Wider implications of the findings: This newly developed model will allow us to better characterize the interactions between immune cells, endometrium and the implanting embryos. This will provide further insights into human embryo implantation, and potentially lead to novel markers or predictors of implantation failure.
Trial registration number: NA

P-347 Patients with repeated implantation failure and Natural Killer cells immaturity: the efficiency of the endometrial scratching strategy

F. Lamazou[1], P. Oger[1], P. Meicler[1], G. Kerbrat[1], G. Soudre[1], E. Genauzeau[2], V. Maget[1], X. Guettier[1], L. Delaroche[2], N. Ledee[3]

[1]RAMSAY SANTE Hôpital Privé de Parly 2, Reproductive Medecine, Le Chesnay, France ;
[2]Centre de Biologie Medicale Hôpital Prive de Parly 2, ART laboratory, Le Chesnay, France ;
[3]MatriceLab innove pépinière paris santé cochin hôpital cochin paris, ART Center, Paris, France

Study question: Is the scratching an efficient treatment for patients with history of repeated implantation failure (RIF) associated with a uterine Natural Killer (uNK) cells immaturity?
Summary answer: The endometrial scratching performed in the mid luteal phase may increase live birth rates in patients with RIF and uNK cells immaturity
What is known already: Twenty to 25% of RIF patients have been described to have uNK cells immaturity. When performed in the mid-luteal phase, the endometrial scratching stimulates the local expression of IL-15 in the mid-luteal phase of the following cycle. The IL-15 secretion allows the uNK cells maturity which is essential for an effective implantation since they regulate local angiogenesis and invasion of spiral arteries.
Study design, size, duration: This is a single-center observational study performed from January 2016 to March 2019. The institutional review board approved this study and the patients'consent to the use of their data was prior obtained.
Participants/materials, setting, methods: Patients with RIF history (>4 top blastocysts) and normal explorations were proposed an endometrial biopsy to analyse their uterine immune profile. The endometrial immune profile documented the ratio of IL-15/Fn-14 mRNA and the IL-18/TWEAK mRNA ratio. Patients with an uNK cells immaturity were included. A scratching in the mid-luteal phase was performed before a new fresh or a frozen-thawed blastocyst transfer in the following cycle. The main endpoints were the implantation and live birth rates.
Main results and the role of chance: Sixty-nine RIF patients with an uNK cells immaturity on their uterine immune profile were included in this study. They were 35.1 ± 1.2 [LD1] years old and had a median AMH level of 2.6 ng/ml [1,2-4.2]. Forty-four patients (64%) received an endometrial scratching in the mid-luteal phase followed by an embryo transfer. The mean number of blastocysts transferred was 2.2 [1-2]. The implantation rate was 40% and 54.4% of these couples achieved a live birth. The rate of spontaneous abortion reached 11.2%.
Limitations, reasons for caution: Despite the bias inherent to a retrospective study and the small number of patients included, these preliminary encouraging results call a prospective randomized controlled trial with a higher number of patients.
Wider implications of the findings: This study suggests that the uterine immune profile can select a population for who the endometrial scratching can be an efficient strategy in RIF situation with an uNK cells immaturity to improve the implantation and live birth rates.
Trial registration number: non applicable

P-348 The remaining cell-free DNA from noninvasive preimplantation genetic test (NIPGT-A) could be used to identify single nucleotide polymorphisms (SNPs) related to the implantation process.

J.G. Franco Jr., M.D.[1,2], L.D. Vagnini[2], C.G. Petersen[1,2], A. Renzi[2], M. Canas[2], B. Petersen[1,2], A.L. Mauri[1,2], M. Mattila[1], J. Ricci[1], A. Nicoletti[1], C. Zamara[1], A.H. Oliani[3], F. Dieamant[1,2], R. Baruffi[1,2], J.B.A. Oliveira[1,2]

[1]Centre for Human Reproduction Prof. Franco Jr, Research, Ribeirao Preto, Brazil ;
[2]Paulista Center for Diagnosis Research and Training, Research, Ribeirao Preto, Brazil ;

[3]Sao Jose do Rio Preto School of Medicine FAMERP, Research, Sao Jose do Rio Preto, Brazil

Study question: Could the remaining cell-free DNA from the same sample used for NIPGT-A diagnosis also identify SNPs related to the embryonic implantation process?
Summary answer: Regardless of the cell-free DNA concentration after the amplification process used by NIPGT-A technology, the success rate of genotyping was 75%.
What is known already: NIPGT-A is a new technology that uses cell-free DNA present in the spent culture medium of human blastocyst, which reflects its ploidy status. This DNA is an important source of information for the embryo. Since there is a gene pattern signature of endometrial receptivity, several SNPs such as those found in the LIF, VEGF, TP53 and MMP9 genes have been used as markers of the implantation process. However, there are no reports on the possibility of identifying SNPs in embryos using remaining cell-free DNA in culture medium.
Study design, size, duration: This prospective cohort study included 23 samples of remaining cell-free DNA obtained in culture medium on day 5 after embryo culture during NIPGT-A technique (Yikon Genomics). A total of 13 patients participated in this study after the couple's informed consent.
Participants/materials, setting, methods: Cell-free DNA evaluation used the amplified DNA obtained after NIPGT-A technique (Yikon Genomics) and quantified by Qubit fluorometer (Thermo Fisher Scientific). SNPs were evaluated by real-time polymerase chain reaction (PCR) using individual TaqMan® SNP genotyping assays (Thermo Fisher Scientific) for each SNP (LIF rs929271, TP53 rs1042522, VEGF rs3025010, MMP9 rs17576) and TaqPath™ ProAmp™ Master Mix (Thermo Fisher Scientific), following the manufacturer's instructions, on a StepOnePlus™ Realtime PCR System (Thermo Fisher Scientific).
Main results and the role of chance: All cell-free DNA samples in the culture medium had at least one SNP identified regardless of their quantification (Table 1). It was observed that of all 92 genotyping reactions performed, 69 were properly amplified, leading to an accuracy of 75%.
Limitations, reasons for caution: Clinical validation studies are underway to determine the predictive value of this methodology.
Wider implications of the findings: This study showed a novel system (NIPGT- A/SNPs) for embryo tracking. The studied SNPs involved in the implantation process were successfully amplified and genotyped with the remaining cell-free DNA of culture medium after NIPGT-A. In the future, NIPGT-A/SNPs dual evaluation could be an additional tool for embryo selection
Trial registration number: Not Applicable

P-348 Table 1. Main results of SNPs genotyping

SAMPLE ID	DNA [ng/uL]	LIF (G>T)	VEGF (C>T)	TP53 (C>G)	MMP9 (A>G)
1	10.4	*	CC	CC	GG
2	13.4	*	TT	GG	*
3	13.3	GG	CC	*	*
4	18.2	TT	*	*	AA
5	10.2	*	CC	GG	GG
6	9.7	TT	*	GG	*
7	47.0	TT	TT	GG	AA
8	16.8	GG	CT	GG	GG
9	11.7	GG	TT	*	*
10	37.0	TT	TT	CC	AG
11	24.0	TT	TT	CC	AA
12	27.0	TT	TT	GG	GG
13	31.0	TT	TT	GG	GG
14	25.0	TT	TT	*	AA
15	92.0	TT	CT	CC	AA
16	32.0	TT	TT	CC	*
17	24.0	TT	TT	*	AA
18	32.0	TT	CT	CC	AA
19	15.8	*	CT	*	*
20	9.7	*	*	CC	*
21	24.6	TT	TT	GG	GG
22	28.6	GG	CC	GG	AA
23	9.8	GG	TT	*	*

***genotyping failure**

P-349 Serum beta human chorionic gonadotropin (β-hCG) levels 13 to 14 days after embryo transfer (ET) and the predictability of pregnancy outcome in IVF cycles

L. Luque[1], N. Ruiz[2], Á. Linares[3], J. Bartolomé[3], J.A. Ortíz[3], R. Bernabéu[4]

[1]Instituto Bernabeu Albacete, Reproductive Medicine, ALBACETE, Spain ;

[2]Instituto Bernabeu Albacete, Embriologist, Albacete, Spain ;

[3]Instituto Bernabeu Albacete, Embriologist, Albacete, Spain ;

[4]Instituto Bernabeu, Reporoductive Medicine, Alicante, Spain

Study question: Is the first β-hCG value predictive of pregnancy outcome in IVF cycles?
Summary answer: Pregnancy viability can be predicted by the levels of early β-hCG in IVF cycles.
What is known already: There is an international agreement to consider> 5 mUI / ml of β-hCG in blood as the positive predictive value after 10-15 days post-transfer, this being the standardized reference level for a pregnancy diagnosis. The subsequent evolution is very variable according to the initial value. Levels past 50 mIU / ml are considered to have a good prognosis. Low levels are related, in most cases, with non-evolutionary pregnancies. There is a growing interest in establishing ranges that can be related to the subsequent evolution of pregnancy.
Study design, size, duration: Retrospective evaluation of a cohort of women with a positive result of serum β-hCG in IVF-ET cycles in a private fertility center. The sample includes 196 cycles performed between July 2017 and January 2020. The study explores the correlation between positive early serum β-hCG (mUI/ml) and pregnancy outcome in terms of: biochemical pregnancy (BP), early pregnancy loss (EPL), ongoing pregnancy (OP) and live birth (LB) rate.
Participants/materials, setting, methods: Women who carried out β-hCG determination in blood 13/14 days post egg retrieval in fresh and frozen transfer, respectively. Blood levels were measured using enzyme-linked fluorescent immunoassay (ELFA), with Minividas from Biomérieux analyzer. Own and donor egg cycles were included. The link between variables of IVF-cycle and serum β-hCG was evaluated using U-Mann-Whitney or Kruskal-Wallis. The β-hCG value as a predictor of the clinical results of IVF was evaluated by a binary logistic regression (SPSSv20.0).
Main results and the role of chance: The mean patient age was 39.1 years. Average blood levels of β-hCG were 201.74 ± 339.26 mIU/mL. The total clinical rates were: 28.1% BP, 33.7% EPL, 71.9 OP y 42.9 % LR. Statistically significant differences were found between β-hCG levels in fresh embryo cycles (170.6) and frozen (228.7) (p=0.006). No statistically significant differences were seen between β-hCG values in own egg cycles or donor (p=0.954) nor with natural endometrial preparation or with transdermal estrogen or oral and vaginal progesterone in frozen transfers (p=0.099). No link was found between β-hCG levels and the day of embryo development. With regards to clinical prognosis, a direct relation was found between β-hCG levels and rates of BP(OR=0.981, IC 95% [0.974-0.988], p<0.001), OP (OR=1.02, IC 95% [1.012-1.027], p<0.001) y LB ido vivo (OR=1.003, IC 95% [1.001-1.005], p<0.001). Confounding variables were introduced to avoid bias: maternal age, cycle of own or donor egg, endometrial thickness, natural or HRT cycle, number of embryos transferred and fresh or frozen embryos. When analyzed in quartile the value of β-hCG,

the rate of LB was: first quartile (hCG38.5) 6.5%, second (hCG: 35.5-120-8) 47.4%, third (120.8-214.5) 54.1%, fourth (>214.5) of74.3% (p<0.001).
Limitations, reasons for caution: Larger prospective studies including homogeneous cohorts are needed in order to corroborate our initial results.
Wider implications of the findings: Early serum β-hCG values constitute a potential biomarker for pregnancy outcome in assisted reproduction IVF cycles.
Trial registration number: Not applicable

P-350 Endometrial assessment and personalised plan in the treatment of patients with unexplained repeated embryo implantation failure: initial outcomes from a dedicated Implantation Clinic

M. Rahmati[1], A. Ripanelli[1], M. Ollila[1], S. Nair[1], A. Gill[1], R. Balet[1], E. Kuan[1], K. Ahuja[1], N. Macklon[1]

[1]London Women's Clinic, Reproductive Medicine, London, United Kingdom

Study question: Can targeted treatment based on endometrial assessment produce satisfactory outcomes in women who have suffered unexplained repeated embryo implantation failure?
Summary answer: A personalised treatment plan based on endometrial diagnostics leads to good clinical outcomes and may represent an improvement on current largely empirical management approaches.
What is known already: Unexplained recurrent embryo implantation failure (RIF) represents a major challenge in reproductive medicine. The current management of RIF is largely based on empirical treatments aim at improving endometrial receptivity. However, the emergence of tests of different aspects of endometrial function provides the opportunity to direct therapies to address underlying pathologies.
Study design, size, duration: A prospective cohort follow up study analysed the endometrial testing results and clinical outcomes achieved from a referral clinic set up in June 2018, dedicated to the investigation and treatment of patients with unexplained repeated embryo implantation failure, who had at least three high quality blastocysts transferred without success.
Participants/materials, setting, methods: Timed endometrial biopsy was performed after 5 days of luteal support in a hormone substituted cycle. Biopsies were subject to endometrial dating by gene expression (ERA test, iGenomix, Valencia) and to endometrial immune profiling including the recruitment and activation of the uterine Natural Killer cells (MLI test, Matrice Lab Innove, Paris). Based on the results, a management plan focusing on optimizing endometrial preparation and embryo transfer was proposed to the referring clinician.
Main results and the role of chance: 170 patients were referred to our unit. 112 patients with an average of 4.3 blastocysts previously transferred underwent investigation. 91% of the patients tested revealed at least one abnormal test outcome. For 70% of the patients, the ERA test showed a non-receptive endometrium. For 75% of the patients, the MLI test found an endometrial immune dysregulation. No correlation was observed between the results of the two test profiles. The outcome of the first attempt implementing a personalised plan showed a pregnancy rate of 52% and an ongoing pregnancy rate of 37% after the first trimester, per transferred blastocyst. Those who did not achieve a subsequent implantation showed a higher prevalence of normal endometrial profiling compared to those who conceived on the implemented treatment plan.
Limitations, reasons for caution: In order to assess the added value of personalised a treatment plan, outcomes need to be compared prospectively with a matched control group who are managed without implementation of a personalised plan.
Wider implications of the findings: Our study suggests that treatment based on endometrial diagnostics might improve outcomes compared with empirical management. This approach provides the opportunity to carry out randomized controlled trials of targeted rather than blind interventions.
Trial registration number: Not Applicable

P-351 First clinical outcomes after personalized embryo transfer using the new endometrial receptivity test in recurrent implantation failure patients

Y. Ohara[1], H. Matsubayashi[1,2], K. Yamaguchi[1,2], K. Kitaya[2], M. Doshida[1], T. Takeuchi[1], T. Ishikawa[1,2]

[1]Reproduction Clinic Tokyo, Department of reproductive medicine, Tokyo, Japan ;
[2]Reproduction Clinic Osaka, Department of reproductive medicine, Osaka, Japan

Study question: Is the new ERPeak[SM] endometrial receptivity test useful for patients with recurrent implantation failure (RIF)?
Summary answer: A displaced window of implantation detected by ERPeak[SM] test was frequently observed in RIF patients. Personalized embryo transfer after ERPeak[SM] testing may improve pregnancy outcomes.
What is known already: Lack of synchronization between an embryo and the timing of endometrial receptivity is thought to be a cause of RIF. Therefore, correctly identifying the window of implantation (WOI) is essential for maximizing the effectiveness of assisted reproduction treatments. While the widespread endometrial receptivity assay (ERA) uses microarray analysis to determine the transcriptomic profile of 238 genes, the ERPeak[SM] test analyses 48 genes by RT-qPCR, a methodology that has been demonstrated to have the highest sensitivity, widest dynamic range and least bias for gene expression analysis. This is the first report of clinical outcomes using ERPeak[SM] testing for RIF patients.
Study design, size, duration: A retrospective review was performed for 137 patients who underwent ERPeak[SM] testing in our clinic between April and October 2019. A total of 119 patients under 45 years old, who had 2 or more failed embryo transfers and underwent personalized embryo transfer (pET) after ERPeak[SM] testing, participated in this study. A hormone replacement cycle had been performed for all patients. The first day of progesterone administration was defined as P+0.
Participants/materials, setting, methods: An endometrial biopsy was performed on day P+5 in an HRT cycle. After the ERPeak[SM] test result was given as receptive, pre-receptive or post-receptive, pET was performed in a subsequent cycle on the day where the ERPeak[SM] test indicated optimal receptivity. In receptive cases, we also considered embryonic developmental speed to set the day of transfer: blastocyst grade 3,4,5 and 6 were transferred on day P+5, P+5.5, P+6.0 and P+6.5, respectively.
Main results and the role of chance: Of 119 RIF patients (average age, 38.8 years), ERPeak[SM] testing showed a shifted WOI result in 50 patients (42.0%) and a receptive (R) result in 69 patients (58.0%). In the shifted WOI group, 66.0% (33/50) indicated a pre-receptive state and 34.0% (17/50) resulted in post-receptive state. After pET for shifted WOI patients, we found that the pregnancy rate and implantation rate were similar between shifted WOI and R patients (46.0% vs. 41.5% and 23.8% vs. 19.3%, respectively), which is consistent with previous studies of pET based on the ERA test. pET for shifted WOI patients showed similar pregnancy rate (42.9%, 47.4% and 47.1%) and implantation rate (20.0%, 28.6% and 21.9%) stratified by patients' age (<38, 39-41, 42-45 years old), respectively. Among R patients, 16 patients received pET in consideration with embryonic developmental speed and 53 patients without such consideration. the pregnancy rate and implantation rate of the former group were higher (62.5% vs. 41.5% and 34.5% vs. 19.3%) compared to the latter, although differences were not statistically significant.
Limitations, reasons for caution: There are several limitations, including small sample size and lack of control group to compare clinical outcomes among RIF patients who are not treated by pET with the ERPeak[SM] test. Embryos were selected for transfer by morphology alone, rather than chromosomal screening, which may have affected the clinical outcome.
Wider implications of the findings: A shifted WOI detected by ERPeak[SM] was frequently observed in RIF patients. pET for shifted WOI patients after ERPeak[SM] testing was consistent with ERA for pregnancy outcomes regardless of patients' age. pET for R patients in consideration with embryonic developmental speed may also improve pregnancy outcomes. Further studies are required.
Trial registration number: not applicable

P-352 Retrospective evaluation of diagnostic usefulness of endometrial lymphocyte subpopulations quantification: influence in endometrial receptivity and in pregnancy outcome in the setting of assisted reproduction techniques

D. Valero Hervás[1], J. Sarasa[2], M. Enciso[2], L. Agúndez[1], J. Aizpurua[3]

[1]iGLS, Immunology, Alicante, Spain ;
[2]iGLS, Research and Development, Alicante, Spain ;
[3]IVF Spain, Gynaecology, Alicante, Spain

Study question: Is the distribution of lymphocyte populations in the endometrium associated with endometrial receptivity and the appearance of early complications during pregnancy?
Summary answer: Endometrial lymphocyte populations are associated with endometrial receptivity status and can be a useful indicator for the early diagnosis of adverse reproductive outcomes.
What is known already: During pregnancy, a series of crucial adaptations occur in the maternal immune system, aimed at ensuring correct embryo implantation and development, while avoiding recognition of the embryo as foreign tissue that could lead to its rejection. Recent studies have shown that disturbances in this balance may lead to pregnancy complications, so the achievement of a successful pregnancy requires maternal and fetal cells coexistence in a tightly regulated balance. In this study, the different immune populations' profiles present in the endometrium and their association with different infertility conditions have been evaluated.
Study design, size, duration: This is a retrospective study analysing immune populations distribution in endometrial tissue and assisted reproduction techniques (ART) outcomes of patients undergoing immune status assessment under different clinical suspicions (recurrent implantation failure or/and miscarriage and primary infertility). Immune profiles were analysed in 239 patients between October 2018 and December 2019. These profiles were compared according to the day of the endometrial cycle in which the sample was obtained and the receptivity status measured by ER Map®.
Participants/materials, setting, methods: Patients referred for endometrial function evaluation were included in the study. Biopsy samples were obtained in hormone replacement therapy (HRT). ImMap® test consisting on the evaluation of the percentage of different Natural Killer (NK) cell phenotypes, Th1, Th2, Th17 and regulatory T lymphocytes, and B1a lymphocytes by means of flow cytometry was performed in all cases. The profiles identified were evaluated according to the available values of normality stated in the scientific literature.
Main results and the role of chance: Our study showed differences in population dynamics depending on the day of the cycle in which the biopsy was performed. The samples obtained between days P4+5 and P4+6 showed significant enrichment in NK cells compared to those taken further away from the theoretical implantation window (WOI) (T-test: 35.67% vs 26.37%; p=0.016). These differences were confirmed analysing exclusively those endometria classified as receptive using ER Map® technology (T-test: 36.02% vs 25.59%; p=0.039), and a decrease in the Tc cells was also identified (T-test: 54.97% vs 48.82%; p=0.042). Furthermore, the receptivity stratification also showed that the lack of this predominance of the NK cells is associated with a post-receptive endometrial state (T-test: 36.39% vs 11.47%; p=0.028). Similarly, a receptive endometrium would be characterized by a lower proportion of the lymphoid group characterized by CD45 (Man-Whitney test: p=0.0001), compared with those non-receptive. In the context of the clinical outcomes, the results show that in women with a WOI between P4+5 and P4+6 who suffered from early abortions, had a lower proportion of activated NK cells when compared to those women who did not develop any adverse effects (p=0.034).
Limitations, reasons for caution: Our study reveals differences in immune populations dynamics with potential usefulness in predicting pregnancy evolution. However, the context and type of sample evaluated hinders the establishment of normality ranges, so to understand the extent of its usefulness it would be necessary to carry out clinical trials including healthy controlled women.
Wider implications of the findings: The application of ImMap® assessment as a routine tool for identifying abnormal immune profiles would lead to the optimal and appropriate medical intervention, in order to prevent or minimize the appearance of adverse outcomes and improve effectively the ART outcomes, and the life-quality of couples with a reproductive desire.
Trial registration number: Not applicable

P-353 DNA extraction protocol optimized for low-biomass samples are essential for accurate profiling of the intra-uterine microbiota.

J. Centelles Lodeiro[1]

[1]*KU Leuven, VIB-KU Leuven Center for Microbiology, Leuven, Belgium*

Study question: Does the DNA extraction protocol affect the accuracy of low biomass intra-uterine microbiota profiling?
Summary answer: A DNA extraction protocol designed for low-biomass samples allows to increase intra-uterine microbiota profiles quality.
What is known already: In recent years, the view of a sterile uterus is being challenged, there is a growing interest in understanding the potential role of microbiota of the female upper reproductive tract in processes leading to a healthy pregnancy. Indeed, several publications have shown preliminary associations between the intra-uterine microbiota and the outcome of pregnancy. However, samples containing low microbial biomass, such as intra-uterine samples, are especially susceptible to contamination by DNA and/or cross-contamination between samples, which can easily dominate the obtained microbiota profiles and lead to erroneous interpretation of results.
Study design, size, duration: Bacterial DNA from intra-uterine biological replicate samples (n=38x2) was extracted using two methods: automated extraction using a standardized kit and manual extraction in a room specially designed to handle low-biomass samples. Technical and procedural negative controls were taken along the process (n=33).
Participants/materials, setting, methods: Intra-uterine samples were retrieved using embryo transfer catheters with a sterile bridging tube, flash-frozen in liquid nitrogen and stored at -80C. DNA was isolated using a Tecan Nucleic Acid Extraction-Platform (MagAttract PowerMicrobiome DNA/RNA kit [Qiagen]) or using a low-biomass-optimized manual extraction (Allprep DNA/RNA kit [Qiagen]). Microbiota profiles were obtained by 16S rRNA amplicon sequencing (V4) using the gold-standard DADA2 pipeline, and analysed using constrained principal coordinates analysis at genus level (cPCoA).
Main results and the role of chance: Biological replicate samples (N=38x2) of the intra-uterine microbiota were subjected to two extraction protocols to evaluate the importance of protocol optimization for these low-biomass samples. The extraction protocol explained 27% of the variation in intra-uterine microbiota profiles (n=76, db-RDA, R2=0.27, adjP=0.0002). Microbial diversity was higher with the DNA extraction protocol optimized for low-biomass samples as compared to the standard protocol (n=76, Simpson diversity index, Wilcoxon rank-sum, r=0.0028, P=3.61E-09). We tracked, in the negative controls of each protocol, a total of 119 genera as contaminants, 13 of which were common to both protocols. We thus identified 90 genera of trustworthy biological sampling origin (non-contaminants) in the standard protocol, compared to 136 in the optimized protocol. These genera were used to calculate the amount of sequencing data generated per biological sample that is of trustworthy origin. With the standard protocol, only 21% of the intra-uterine samples achieved the minimum threshold of 1000 trustworthy sequencing reads, while 79% of the biological samples passed that threshold with the optimized protocol.
Limitations, reasons for caution: The negative controls used in this analysis focused on the evaluation of contaminants during DNA extraction and sequencing. The addition of negative controls for the sampling procedure (empty sampling devices) would give additional information on potential DNA contaminants in devices used for sampling.
Wider implications of the findings: Applying 16S rRNA amplicon sequencing on low-biomass intra-uterine samples is a two-edged sword since its efficiency for detecting microbial DNA also makes it highly sensitive to contaminant DNA and to cross-contamination. Ignoring this drawback can lead to erroneous interpretation of microbiota profiles and their role in human reproductive health.
Trial registration number: NCT03105453

P-354 Placental morphology remains unaffected by addition of GM-CSF during pre-implantation development

V. Nordhoff[1], **L. Ogoniak**[1], **T. Pock**[1], **K. Schulte**[2], **S. Schlatt**[1], **M. Boiani**[3]

[1]*University Hospital of Muenster, Centre of Reproductive Medicine and Andrology, Münster, Germany ;*
[2]*University of Muenster, Central Animal Facility of the Faculty of Medicine, Münster, Germany ;*
[3]*University of Münster, Max Planck Institute for Molecular Biomedicine, Münster, Germany*

Study question: Does GM-CSF supplemented during pre-implantation development have an influence on fetal growth or placenta development?
Summary answer: GM-CSF had an effect on fetal weight but placenta morphology was not affected by different concentrations of GM-CSF.

What is known already: The growth factor granulocyte macrophage colony-stimulating factor (GM-CSF) is used in ART for preimplantation embryo cultivation to increase survival of embryos up to week 12, especially in women with previous miscarriages. Naturally, GM-CSF is produced by the endometrium during early gestation. The corresponding receptors can be found in the trophoblast cells of the early embryo. After implantation trophoblast cells establish the connection of the embryo with the maternal tissue and the placenta forms. The mature placenta consists of the fetal layers labyrinth and spongiotrophoblast and the maternal decidua.
Study design, size, duration: Female mice (B6C3F1, 5-10 weeks old) were superovulated by injection of 5 IU PMSG and 10 IU hCG and mated to males (C57BL/6J). Zygotes were isolated on day 0.5 post coitum, pooled and randomly allocated to KSOM(aa) with 0, 2 and 10 ng/mL mGM-CSF. Medium refreshment was applied on day 2.5. Embryos were transferred to foster mothers and the developing fetuses and placentas were dissected at day 13.5 of gestation.
Participants/materials, setting, methods: In total 48 placentas and fetuses could be collected of which 41 placentas were appropriate for analysis: 13 placentas in the control group (KSOM(aa)), 15 in the group with 2 ng/mL GM-CSF and 13 in the group with 10 ng/mL GM-CSF. Tissues were collected to measure fetus weight and length and placenta weight. Placentas were embedded, cut and stained by PAS-reaction. Area and diameter of the single placenta layers were measured from 6 sections each.
Main results and the role of chance: Fetal weight was significantly higher after culture in medium containing 2 ng/mL GM-CSF compared to the control medium (mean values: 0ng/mL=0.17g; 2ng/mL=0.21g; 10ng/mL=0.18g; P-value <0.05). No difference was found between placenta weight in different concentrations of GM-CSF (mean values: 0 ng/mL=0.115 g; 2ng/mL=0.122 g; 10ng/mL=0.122 g). Values for placenta layer areas and diameters were similar in each group, as well as the whole placental area (mean values: 0 ng/mL=10.01mm^2 g; 2ng/mL=10.49mm^2 g; 10ng/mL=10.65mm^2 g) and diameter (mean values: 0 ng/mL=2.05mm^2; 2ng/mL=2.19mm^2 g; 10ng/mL=2.29mm^2). A significant correlation was found between body length and weight of the fetuses (R2 = 0.3869). Additionally, fetus length and placenta weight were associated as was placenta area and placenta weight. Whole placenta area and also the single layers (labyrinth, spongiotrophoblast and decidua) and their proportions were similar among all groups.
Limitations, reasons for caution: A limitation of this study is the use of an animal model and the number of examined placentas. Data cannot be transferred 1:1 to the human situation. Further studies should investigate the effect of GM-CSF during several developmental steps in the development of either the embryo or the fetus.
Wider implications of the findings: GM-CSF had an influence on fetus weight but not on placenta weight or morphology. Although it seems that this growth factor has no major influence during culture, still our data create awareness for the general use of growth factors in ART culture media.
Trial registration number: not applicable

P-355 Evaluation of ER Map® test to predict endometrial transcriptomic signatures associated to different infertility conditions and ART outcomes.

J. Sarasa Marcuello[1], J. Aizpurua[2], B. Rodriguez-Estrada[1], I. Jurado[1], M. Ferrández[1], A.B. Climent[3], M. Enciso[1]

[1]iGLS, Research and Devolopment, Alicante, Spain ;
[2]IVF Spain, Reproductive Medicine, Alicante, Spain ;
[3]IVF Spain, Statistics, Alicante, Spain

Study question: Is it possible to identify endometrial transcriptomic signatures with clinical significance using the molecular tool ER Map®?
Summary answer: ER Map® gene expression analysis is able to predict infertility conditions and ART outcomes with clinical significance.
What is known already: Transcriptomic analyses have been proposed in many fields of medicine as powerful tools to identify pathological conditions. In reproductive medicine, gene expression analysis of endometrial tissue has been recognized as an effective approach for endometrial receptivity evaluation. ER Map® is a molecular diagnostic tool able to accurately predict the window of implantation (WOI) by analysing the expression profile of 40 genes by high-throughput RT-qPCR. Other conditions of altered endometrial function beyond receptivity could be responsible of failed ART results. In this study we aim to identify WOI transcriptomic signatures with clinical significance associated to different infertility conditions and ART outcomes.
Study design, size, duration: This is a retrospective study analysing transcriptomic signatures, infertility conditions and ART outcomes of 1095 patients undergoing endometrial receptivity assessment by ER Map® between March 2016 and January 2020. Results obtained when embryo transfers were scheduled on the moment of endometrial receptivity (WOI timeframe) were analysed. Prediction models of gene expression profiles of patients with different infertility conditions and ART outcomes were evaluated.
Participants/materials, setting, methods: A control group of healthy egg-donors and patients referred for ER Map® analysis for a variety of reasons including implantation failure (RIF) and recurrent miscarriage (RM) were included in the study. ER Map® gene expression analysis of endometrial biopsies obtained in HRT cycles at P4+5.5 was performed by qRT-PCR (Biomark-HD, Fluidigm). Transcriptomic profiles from donors, different infertility conditions (RIF, RM) and ART outcomes (successful implantation, biochemical pregnancy and clinical pregnancy) were evaluated by discriminant analysis.
Main results and the role of chance: We have identified WOI transcriptomic signatures predictive of recurrent miscarriage (RM) and implantation failure (RIF). In the RM group the overall accuracy prediction in the training set is 100%, all 37 RM patients and 9 controls were classified in their respective groups. Leave-one-out cross-validation presented a positive predictive value (PPV) of 86.67%. In the RIF group the overall accuracy prediction in the training set is 99.2%, only 2 out of 235 RIF patients were misclassified as controls. Leave-one-out cross-validation presented a PPV of 99.57%.
Analysis of ART outcomes in our cohort showed that 81.64% (894/1095) of patients achieved implantation, of which 9.96% (89/894) had a biochemical pregnancy and 73.52% (805/1095) continued to have clinical pregnancies. Analysis of the gene expression profiles allowed the identification of a WOI signature predictive of successful outcome (clinical pregnancy) and two WOI signatures predictive of negative reproductive outcomes (implantation failure and biochemical pregnancy). The group of samples identified to present a positive outcome signature had an implantation rate of 91.95%, a biochemical pregnancy rate of 3.43% and a clinical pregnancy rate of 88.52%. These rates are significantly different ($p<0.001$) than the ones obtained in the group of samples identified to present negative outcomes signatures.
Limitations, reasons for caution: ER Map® test can serve as a valuable tool to improve the diagnosis of endometrial function and anticipate ART outcomes in sub-fertile patients, however, other types of investigations aimed to determine the therapeutic options for patients at risk of negative reproductive outcomes will also be necessary.
Wider implications of the findings: The ability of ER Map® for the prediction of cases at risk of negative reproductive outcomes offers a comprehensive method for the diagnosis of endometrial function and infertility. This tool may open new research lines to understand endometrial pathology and develop new treatment strategies.
Trial registration number: Not applicable

P-356 Whole-genome sequencing of embryos from pregnancy loss identifies putatively detrimental genomic variants

S. Bonaiuto[1], I. Di Biase[2], V. Aleotti[3], A. Ravaei[3], G. Damaggio[1], M. Chierici[4], P. Madhuri[5], A. Qasim[5], C. Furlanello[4], E. Garrison[6], N. Soranzo[7], A. Capalbo[8], R. Michele[3], S. Di Biase[2], V. Colonna[1]

[1]Consiglio Nazionale delle Ricerche, Istituto di Genetica e Biofisica, Napoli, Italy ;
[2]Merigen Research s.r.l., Research & Development, Naples, Italy ;
[3]University of Ferrara, Department of Environmental- Biological and Pharmaceutical Sciences and Technologies, Ferrara, Italy ;
[4]Fondazione Bruno Kessler, Predictive Models for Biomedicine & Environment, Trento, Italy ;
[5]Monash University Malaysia, School of Science, Jalan Lagoon Selatan, Malaysia ;
[6]University of California Santa Cruz, UC Santa Cruz Genomics Institute, Santa Cruz, U.S.A. ;
[7]Wellcome Sanger Institute, Human Genetics, Hinxton, United Kingdom ;
[8]Igenomix, Igenomix, Marostica, Italy

Study question: Do whole-genome sequences of embryos from recurrent pregnancy loss clarify the impact of genetic variants of small-size and/or located in non-coding regulatory regions?

Summary answer: Whole-genome analysis of embryonic DNA from pregnancy loss successfully identifies putatively detrimental genomic variants

What is known already: Pregnancy Loss (PL), the spontaneous demise of pregnancy before 24 weeks of gestation, occurs in 10-15% of pregnancies. PL is often the result of chromosomal aneuploidies of the gametes but it can also have non-random genetic causes like small mutations (SNPs and indels), both de-novo or inherited from parents. Comparative genomic hybridization (CGH) detects variants of several thousand base pairs while targeted resequencing resolves point mutations. Both are currently the most accurate methods for the genetic analysis of PL, but are not sensitive to small variants (CGH), or do not target those located in non-coding regulatory regions (targeted resequencing).

Study design, size, duration: Our aim is to identify small-size genetic variants likely to cause PL using a predictive model integrating whole-genome sequence data with functional annotations and gene networks relevant to embryonic development. Seventy women, mostly European (82.7%) diagnosed with first (n=39, av.age 28.9) or recurrent (n=31, av.age 39.0) miscarriage were recruited by the University of Ferrara from 2017 to 2019. Ethical committee approval is from Emilia-Romagna CE/FE (#170475).

Participants/materials, setting, methods: Fetal DNA extracted from chorionic villi was used to exclude samples with aneuploidies using both CGH and shallow sequencing of random genomic regions. Euploid samples were whole-genome sequenced at high-coverage. Variants were called against the reference genome GRCh38 with Freebayes. Variants were annotated with a custom script that integrates information from Ensembl99 with publicly available manually curated lists of genes associated with embryonic development, miscarriages, lethality, cell cycle. The code is available on gitHub (ezcn/grep)

Main results and the role of chance: We understood the requirements to scale-up the project and obtained initial results from the analysis of these genomic sequences. We determined that shallow sequencing of random genomic regions is more efficient compared to CGH in detecting aneuploidies in samples with poor quality DNA. We estimated that 20% of collected samples are suitable for sequencing, the rest presenting aneuploidies or quality issues and maternal contamination. Sequenced samples have on average 4M high-quality variable sites that were annotated with information on gene content and functional consequences. In the autosomes 4.3k variants are ranked as having a high deleterious impact according to Ensembl, 69.1k moderate, and 111k low. After filtering based on the combination of several criteria, such as allele frequency in the general population, homozygosity, impact of the allele with consequences we identify a number of putatively detrimental mutations. Three samples carry two homozygous missense mutations in the same exon of the AHNAK2 cancer-related gene, which codes for a cytoplasmic nucleoprotein. Five samples share the same stop gain mutation in the RPTOR gene, a component of a signaling pathway that regulates cell growth and survival, and autophagy in response to nutrient and hormonal signals.

Limitations, reasons for caution: While encouraging, these first results needs to be corroborated with a more comprehensive analysis that fully implement a predictive model. Results will be validated in a biobank of embryonic sequence data from PL.

Wider implications of the findings: We demonstrated that whole-genome sequencing can help to clarify the causes of RPL. The pilot study described here has discovered plausible candidate RPL-associated variants and provides essential indications for the realization of a larger study.

Trial registration number: not applicable

P-357 Assessment of pH at the surface of the endometrium of the uterine fundus is a novel marker of chronic endometritis

T. Endo[1], T. Baba[2], E. Itabashi[3], H. Honnma[4], H. Henmi[5], U. Ikeda[5], T. Kiya[6], T. Saito[7], A. Azumaguchi[8], M. Fujii[3]

[1]Sapporo Medical University, Obs & Gynae Dept., Sapporo- Hokkaido, Japan ;
[2]Sapporo Medical University, Obstetrics and Gynecology, Sapporo, Japan ;
[3]Tokeidai Memorial Hospital, Obstetrics and Gynecology, Sapporo, Japan ;
[4]Sapporo ART Clinic, Obstetrics and Gynecology, Sapporo, Japan ;
[5]Tonan Hospital, Obstetrics and Gynecology, Sapporo, Japan ;
[6]ENA Aazabu ART Clinic, Obstetics and Gynecology, Sapporo, Japan ;
[7]Sapporo Medical University, Obstetriics and Gynecology, Sapporo, Japan ;
[8]Sapporo Endometrium Research, Obstetrics and Gynecology, Sapporo, Japan

Study question: Can assessment of pH at the surface of the endometrium of the uterine fundus distinguish the condition of chronic endometritis(CE) from the normal condition?

Summary answer: Assessment of the pH at the surface of the endometrium of the uterine fundus is thought to be an excellent novel marker of chronic endometritis.

What is known already: CE is a state of persistent inflamation in the endometrium caused by common bacterial pathogens. It is reported to be associated with reproductive failure. Endometrial plasma cells (CD138 immunostainined cells) are accepted as the standard criterion to establish a diagnosis of CE. Recently, sequencing of the 16S rebosomal RNA revealed that lactobacillus species are abundant in the uterine cavity in women without CE, and much less abundant in CE patients. However, the effects of lactobacillus in the endometrium are unknown. Uterine fluid pH in both CE and non-CE is reported to be around 7.2 (Moreno et al.,2016).

Study design, size, duration: We tried to determine whether assessment of the pH of the endometrium could be a new marker of chronic endotmetritis. Asymptomatic women (N=56) with recurrent implantation failure or recurrent miscarriage were recruited. Chronic endometritis was diagnosed using CD138 immunostaining, and the uterine microbioma by using sequensing of the 16S ribosomal RNA gene. We checked uterine pH in both CE and non-CE. This study was performed from June 1 until December 31,2019.

Participants/materials, setting, methods: Participants were recruited from the infertility clinic of Tokeidai Memorial Hospital. Plasma cells in the stroma of endometrium were indentified by means of immunohistochemistry staining. Sequencing of 16S rebosomal RNA gene in the endometrial fluid was done for detecting bacteria. Endometrial surface pH was examined directly at the endometrial surface using a Portable pH Meter (Japan Asch Inc., Japan) that has been utilized for the esophageal mucosa of esophagitis (Journal of Gastroenterology, Japan, 1990).

Main results and the role of chance: We tried to examine some markers of CE in a total of 56 patients with reproductive failure who gave informed consent. We first screened for CE using our criteria following 2 criteria reported by Liu et al.(Fertil Steril, 2019), >5.15 CD138 stained cell/10mm2 or <85% lactobacillus in the Endometrial Microbioma Test (Varinos Inc., Japan). The endometrial pHs of the follicular phase and luteal phase in non-CE were 5.34±1.00(mean±SD)(n=8) and 5.43±0.97(n=13), respectively. This difference was not significant. The endometrial pH of CE, 6.23±0.65(n=35), was significantly different from that of non-CE, 5.61±0.74 (n=21)(P<0.01). The endometrial pH of CE (CD138 cell number>5.15/10 mm2) was 6.39±0.58(n=18), which was also significantly different from that of non-CE(CD138 cell number <5.15/10mm2), which was 5.41±0.79 (n=20)(p<0.01). The endometrial pH of CE(lactobacillus<85%),was 6.67±0.39(n=17), whcih was significantly different from that of non-CE(lactobacillus >85%), which was 5.58±0.59 (n=15)(p<0.01).

Limitations, reasons for caution: As this study was performed in only 56 female patients with infertility or recurrent miscarriage, a larger number of patients need to be studied for precise evaluation of the usefulluness of the dendometrial pH.

Wider implications of the findings: The endometrial pH was usually acidic, unlike in from Mereno et al.'s report. The endometrial pH of CE was significantly higher than that of non-CE. The endometrial lactobacillus is associated with the endometrial surface pH. Therefore, endometrial pH is thought to be an excellent novel CE maker.

Trial registration number: R1919

P-358 Modelling embryo implantation in vitro using a three-dimensional co-culture system

C. Hill[1], D. Hapangama[1], M. Phelan[2]

[1]University of Liverpool, Women's and Children's Health, Liverpool, United Kingdom ;
[2]University of Liverpool, Institute of Integrative Biology, Liverpool, United Kingdom

Study question: Can we construct a 3D *in vitro* model of endometrial embryo implantation to study early human pregnancy establishment?

Summary answer: We were able to develop a 3D human endometrial co-culture model that accepted surrogate embryo implantation to simulate early human pregnancy.

What is known already: The human and non-human primate endometrium is unique amongst mammals in its ability to decidualise, shed and regenerate. Furthermore, the cellular architecture of human endometrium is found only in higher primates. These fundamental differences make it difficult to model early pregnancy establishment in routinely studied laboratory animals. Therefore, novel models of human embryo-endometrium interactions are needed to fully define the factors involved in successful implantation.
Study design, size, duration: A 3D *in vitro* model of the endometrium was established using endometrial epithelial cell line Ishikawa and a telomerase immortalised human endometrial stromal cell line embedded in a collagen hydrogel. The model was expanded to include primary human endometrial epithelial and stromal cells isolated from freshly collected endometrial biopsies from healthy women not on hormonal medication. Embryo implantation was simulated by attachement of Jeg3 trophoblast spheroids over a ten day period.
Participants/materials, setting, methods: Liverpool Women's Hospital is a tertiary referral centre affiliated to the University Hospital Research Centre. Samples of endometrium was harvested from women undergoing benign gynaecological procedures after obtaining informed consent. Surrogate embryo implantation in 3D culture models was assessed by immunohistochemistry. The metabolomic signature of early pregnancy was assessed by NMR analysis of conditioned medium from endometrium-embryo co-cultures.
Main results and the role of chance: We initially employed human cell lines to develop a 3D construct containing epithelial and stromal cells, and a variety of protocol optimisations were necessary to replicate the different cycle phases and decidualisation process of the endometrium. The optimised conditions for cell lines were further adjusted for primary cells, which demonstrated an enhanced capacity for self-organisation and remodelling of the collagen matrix. Successful decidualisation of the 3D constructs in the presence of oestrogen and progesterone was confirmed by ELISA and immunohistochemistry. Jeg3 spheroids were show to attach to the 3D constructs and interact with the epithelial and stromal elements. Preliminary analysis of the metabolome defined by NMR analysis of the co-culture conditioned medium identified metabolites unique to embryo implantation.
Limitations, reasons for caution: Only the Jeg3 choriocarcinoma cell line was used as a surrogate embryo model, which does not fully capture all stages of blastocyst implantation and invasion (e.g. syncytialisation). Future work should incorporate other cell lines and primary cells to model all elements of trophoblast adhesion, invasion and migration.
Wider implications of the findings: Modelling human embryo-endometrial interactions *in vitro* helps to widen our understanding of implantation. Such information will prove invaluable in many areas of reproductive medicine, such as increasing the success of IVF treatment and defining the causes of recurrent miscarriage.
Trial registration number: not applicable

P-359 A simple scoring system for prediction of early pregnancy loss developed by following 13,977 infertile patients after in vitro fertilization

Y. Ouyang[1], P. Cai[2], X. Li[1]

[1]*Reproductive and genetic hospital of Citic-Xlangya, Imaging Department, Changsha, China ;*
[2]*Central South University, Institute of Reproductive and Stem cell engineering, Changsha, China*

Study question: What is the performance of a simple scoring system to predict early pregnancy loss (EPL) after in vitro fertilization-embryo transfer (IVF-ET)?
Summary answer: A simple scoring system using prospectively collected routine data could be easily used in early pregnancy care and effectively guide subsequent medical plans.
What is known already: The first routine ultrasound scan is commonly arranged on day 28 after ET in most reproductive centers to determine the location and viability of the embryo. The mental pressure associated with a pregnancy of uncertain viability are significant in patients undergoing IVF-ET, thus, a rapid and accurate method to predict the possibility of early miscarriage would be helpful for determining subsequent treatment. Previous mathematical models have combined individual risk factors with reasonable performance. However, a simple scoring system that can be easily implemented in clinical practice has not been described for the prediction of EPL after IVF-ET.
Study design, size, duration: This was a prospective study in a single reproductive centre. The infertile patients included in this study underwent IVF treatment between June 2016 and December 2017.
Participants/materials, setting, methods: A cohort of 13,977 consecutive women undergoing transvaginal ultrasound scan on Day 27–29 after ET were included. Ultrasound measurements and clinical characteristics were recorded. The first trimester pregnancy outcome of these women was noted at 12 weeks of gestation. The cases of first year were used to generate a training sample (7,261) and a simple scoring system was derived from this. The cases of second year were used as a verification sample (6,716).
Main results and the role of chance: There were 12,051 cases with an ongoing pregnancy and 1,926 cases had EPL. Maternal age (MA), gestational sac diameter (GSD), crown-rump length (CRL), embryonic heart rate (HR), yolk sac diameter (YSD) and endometrium thickness (EM) on transfer day finally entered the scoring system after stepwise screening. Points associated with each category of each risk factor were computed and the risks associated with point totals were determined according to the "The Framingham Study risk score system".
The scoring system gave an area under the curve (AUC) of 0.884 (95% confidence interval 0.870-0.899) in the training set. The score totals range from point -8 to 14. When point 5 was chosen as the cutoff value, the predicting risk of EPL was 30.03%, with a sensitivity of 66.87%, specificity of 98.11%, positive predictive value (PPV) of 84.44% and negative predictive value (NPV) of 95.07% in the test set, which performed with a sensitivity of 64.69%, specificity of 98.78%, PPV of 89.87% and NPV of 93.62% in the test set. 94.01% cases of the training sample could be correctly predicted as EPL and in the verification samples, 93.91% of the cases were correctly predicted.
Limitations, reasons for caution: This scoring system was used for easy and quick prediction of EPL with false positive and false negative results and the miscarriage outcome cannot be changed by this system. Smoking variable was a potential omission in the data collection and might further improve the predictive performance if included.
Wider implications of the findings: This simple scoring system incorporates readily available data that are routinely collected in clinical practice and does not rely on complex data entry. The findings could be easily incorporated into early pregnancy care and effectively guide subsequent medical plans.
Trial registration number: not applicable

P-360 The incidence of monozygonic twins in assisted reproduction technology -a retrospective study

X. Feng[1], P. Liu[1]

[1]*Center for Reproductive Medicine, Department of Obstetrics and Gynecology-Peking University Third Hospital, Beijing, China*

Study question: does the incidence of MZT (monozygonic twin) in assisted reproduction techonology higher and what are the risk factors?
Summary answer: this retrospective research shows that blastocyst culture significantly increased the risk of monozygonic twins. However, the age, ICSI or assisted hatching have no signifcant impact.
What is known already: It is known that the incidence of monozygonic twin (MZT) in natural conception is about 0.4%. Previous report has shown that the risk of MZT has doubled after assisted reproductive technology. MZT can lead to miscarrige, increase the incidence of preterm birth and other adverse neonatal outcomes. Therefore, researchers have been take much attention to the incidence and reasons of MZT in assisted reproduction and found that maternal age, ICSI and blastocyst tranplantion may be associated with the happening of MZT.
Study design, size, duration: we conducted a retrospective study in our center based on data from 2014 to 2018. In all, 41538 patients that got pregnancy after IVF were include in this study.
Participants/materials, setting, methods: there were 20048 fresh cyclyes and 11885 frozen cycles included. We analyzed different clinical data (maternal age and stimulation strategy) and IVF strategy such as type of embryos, insemination method, embryo stage at time of ET. All pregnance were confirmed by detection of fetal heart activity . The identification of MZT is identified when more than one fetal poles was visualized in one gestational sac via trans-vaginal ultrasound.For the statistical analysis, SPSS software was used.

Main results and the role of chance: In our clinic center, there were 59 patients who were MZT from 12060 patient who transferred D3 fresh embryos(0.49%). In addition, 9 patients were MZT from 590 patients who transferred fresh blastocyst(1.52%). These two groups have significant difference. In frozen cycles, there were 32 patients who got MZT in 5042 patients who received D3 embryo transfer(0.63%)and 145 patients who got MZT in 7085 ones who received blastocyst embryo transfer(1.96%).The incidence of MZT was significantly lower in D3 ET cycles than that in blastocyst ET cycles. In addition, the incidence of MZT in patients who were more than 35 years old have no significant difference with those who wre no more than 35 years old (fresh cycles: 0.66% vs 0.47; frozen cyclyes:1.17% vs 0.86%). Moreover,we analyzed the correlation of insemination with MZT and found that there were no difference no matter whether ICSI were used or not(0.66% vs 0.57% in ICSI). At last, the manupulation of assisted hybradation have no impact on MZT incidence(1.68% vs 1.23% for AH).
Limitations, reasons for caution: . It should be noticed that more data from randomized trial were required.
Wider implications of the findings: From the data we can found that blasotcyst culture and transfer have been identifed to significantly influence the incidence of MZT. Therefore patients should be noticed of the risk of blastocyst cutlure and tranfer.
Trial registration number: no

P-361 Clinical applications of platelet-rich plasma in poor endometrium patients in IVF practice

O. Feskov[1], Y. Zhylkova[2], A. Feskova[3], O. Blazhko[4], O. Yegunkova[5]

[1]Centre of Human Reproduction Sana-Med, IVF, Kharkiv, Ukraine ;
[2]Center of Reproductive Medicine "Clinic of professor Feskov", IVF Department, Kharkiv, Ukraine ;
[3]Kharkiv National Medical University, Department of Obstetrics and Gynaecology, Kharkiv, Ukraine ;
[4]Feskov Human Reproduction Group, Surrogacy Department, Kharkiv, Ukraine ;
[5]Center of Rproductive Medicine "Clinic of Professor Feskov", Genetic laboratory, Kharkiv, Ukraine

Study question: Does the intrauterus application of platelet-rich plasma (PRP) allow to improve the condition of the endometrium and IVF outcome in repeated implantation failure patients?
Summary answer: Application of PRP in patients with refractory emdometrium resulted in satisfactory endometrial thickness. PRP was effective in IVF patients with repeated implantation failure (P< 0.05).
What is known already: The endometrium plays an important role in achieving optimal outcomes of assisted reproductive technologies. PRP is a novel method that is used in reproductive medicine to improve the IVF outcome. The mechanisms of PRP have not been completely elucidated, but laboratory studies have shown that the high concentration of growth factors in PRP can potentially speed up the healing process. Recently, the intrauterine infusion of PRP has been described as a way to promote endometrial growth and receptivity.
Study design, size, duration: The effect of the intrauterus application of PRP to improve the endometrium thickness in patients with repeated implantation failure (RIF) was studied. The implantation rates were evaluated in RIF patients in IVF programs after PRP procedure. The study's protocol was approved by the Center's IRB.
Participants/materials, setting, methods: In total, PRP group included 32 cycles of 20 patients with the mean age 34.2±3.6 y.o. The control group included 38 cycles of 23 patients with the mean age 35.7±4.2 y.o The studies period was January - December 2019. PRP was infused per intrauterine catheter on the 9th day of hormone replacement therapy cycle and progesterone administration day. All patients were transferred two good-quality blastocysts. T-test and Chi-squared test were used for data analysis.
Main results and the role of chance: After PRP infusion, the average endometrium thickness on day of progesterone administration in PRP group was 8.31±1.18mm, which was significantly thicker than control group (5.87±0.81mm) (Student t-test t=2.13, P = 0.04). The implantation rate and clinical pregnancy rate in PRP group were significantly higher than control group (28.13% vs. 15.79%, P<0.05; 25.0% vs. 10.53%, P<0.05, respectively). There was no difference in cycle cancellation rate in both groups.
Limitations, reasons for caution: The embryo transfer was done in case of the emdometrium thickness not less then 6 mm.
Wider implications of the findings: It is not clear how the intrauterine administration of PRP acts to affect endometrial thickness. Results from studies on the role of endometrial thickness on implantation and live births are contradictory. There is the urge for well-designed randomized studies to improve our knowledge on PRP in basic science.
Trial registration number: No number

P-362 New predictors of early impaired placentation preceding miscarriage before 10 weeks of gestation in IVF pregnancies: a prospective study

A. Ben-Haroush[1], H. Hamutal[2], A. Wertheimer[1], K. Tenenbaum-Gavish[3], Y. Shufaro[1], A. Idelson[3]

[1]Beilinson, IVF and Infertility Unit, Petach - Tikva, Israel ;
[2]TeleMarpe Ltd, Tel Aviv, Tel Aviv, Israel ;
[3]Rabin Medical Center, Obstetrics and Gynecology Ultrasound Unit, Petach Tikva, Israel

Study question: We prospectively evaluated early first-trimester gestational sac and fetal biometry with maternal blood PP13, to predict impaired placentation prior to miscarriage before GA 10 weeks in IVF pregnancies.
Summary answer: In IVF pregnancies with live embryo at first ultrasound scan, high UtA-PI/CRL and PP13/CRL ratios may indicate impaired placentation preceded early pregnancy loss.
What is known already: Fetal loss during the first trimester occurs in 10-20 percent of pregnancies. In a previous study we showed that small CRL in the early first trimester may predict early fetal loss in IVF pregnancies; fetal losse rate was 17.2% in pregnancies with CRL ≤10 percentile compared to 6.6% in the appropriate to GA CRL group (*P*=0.005). Placental protein 13 (PP13), is exclusively expressed in placenta from a very early stage of pregnancy and can be detected in the maternal blood from the fifth week of gestation. Some studies have indicated that damaged placenta may leak high levels of PP13.
Study design, size, duration: Cohort of 115 healthy IVF patients with a singleton viable embryo in early first trimester.
Participants/materials, setting, methods: Calculation of gestational age (GA); ultrasound evaluation of crown-rump length (CRL), mean gestational sac diameter (GSD) and volume (GSV), mean yolk sac diameter (YSD) and volume (YSV); fetal heart rate (FHR), mean uterine arteries pulsatility index (UtA-PI); and maternal blood placental protein 13 (PP13) levels. Patients were divided into three groups by GA; and early miscarriage versus ongoing pregnancy after GA 10 weeks.
Main results and the role of chance: Early fetal loss occurred in 14.8% of patients; miscarriage group had higher discrepancy between calculated and measured GA (*P*<0.001), lower GSD and GSV (*P*=0.005 and *P*=0.02, respectively), significantly different YSD and YSV, lower mean GSD and volume ratios (*P*=0.001 and *P*=0.003, respectively), and higher UtA-PI/CRL ratio with miscarriage at GA of 46-48 days and GA ≥49 days (*P*=0.034 and P=0.026, respectively). PP13/CRL ratio was higher in patients with miscarriage at GA ≥49 days (*P*<0.041).
Limitations, reasons for caution: Small cohort, only IVF pregnancies.
Wider implications of the findings: Possible very early prediction of impaired placentation prior to early pregnancy loss. A larger cohort is needed to further verify the clinical utility of these miscarriage predictions in IVF pregnancies.
Trial registration number: NA

P-363 The Association between Serum Estradiol and Progesterone on the Same Day of FET and the Pregnancy Outcome

K. Rageh[1], A. Barakat[2], N. Barakat[3]

[1]Al-Azhar university, Ob/Gyn., Cairo, Egypt ;
[2]Al-Baraka Fertility Hospital, Ivf, adliya, Bahrain ;
[3]Al- Baraka fertility hospital, lab, adliya, Bahrain

Study question: To investigate the impact of serum E2 and P levels on the same day of embryo transfer on pregnancy outcomes for FET cycles.

Summary answer: The association between serum estradiol and progesterone levels on the same day of FET and the pregnancy outcome is still not proven
What is known already: So far, most studies about FET cycles have focused on embryological factors and the thickness of endometrium, while little attention has been paid to the serum steroid hormone levels on the day of embryo transfer.

Besides endometrial thickness, E2 and P levels are also regularly monitored for endometrial receptivity. However, whether serum E2 and P levels on the day of embryo transfer can serve as an indicator for clinical pregnancy is doubtful. The effect of serum E2 and P levels on the day of embryo transfer is a matter of controversy in the literature and needs further evaluation.
Study design, size, duration: This was a retrospective cohort study (registered at ***clinical trials.gov with ID NCT04114500)***, for 402 FET cycles which conducted in Al- Baraka Fertility Hospital, Manama, Bahrain, between April 2018 and May 2019
Participants/materials, setting, methods: Endometrial preparation for 402 patients received estradiol tab. When end.thickness reached 8 mm or greater, patients were initiated on both vaginal and oral Progest. A depot GnRH agonist was administered in midluteal phase of the preceding cycle. On the same day of FET, serum levels of E2 and P were assessed, Then, transfer of PGT-euploid embryos was performed. 12 days later pregnancy test was assessed, and then ultrasound was scheduled to check the viability
Main results and the role of chance: Serum P and estradiol levels the same day of FET were measured. A multivariable analysis to assess the relationship between serum E2 & P level and pregnancy outcomes was performed, adjusted for confounding variables. Mean E2 value was 931.41 ± 438.65 pg/ml, while mean P value was 8.47 ± 9.4 ng/ml. Progesterone levels were split in terciles: T1: <15 ng/ml; T2: 15–30 ng/ml; T3: > 30 ng/ml. Out of 402 cases, 240 cases had positive pregnancy test (59.7%) while the clinical pregnancy rate was 53.9% (217 cases out of 402) with no correlation between serum (E2, P & E/P ratio) and the pregnancy rate
Limitations, reasons for caution: its retrospective design, which precludes drawing conclusions regarding how to improve pregnancy outcomes in FET patients using serum estradiol and progesterone levels as predictors; also in our study, we didn't evaluate live birth rates (LBR).
Wider implications of the findings: The implantation process is the most vital and the least understood part of reproduction.trying to understand this inigma of implantation in the future we are planning to go for a prospective study using only vaginal progesterone, with evaluation of secondary outcomes e.g. live birth rates (LBR), and spontaneous abortions/biochemical pregnancies.
Trial registration number: NCT04114500

P-364 Expression of vascular endothelial growth factor (VEGF), placental growth factor (PLGF) and insulin-growth like factor 1 (IGF-1) in serum from women undergoing frozen embryo transfer.

X. Guo[1], T.C. Li[1], X. Chen[1]

[1]The Chinese University of Hong Kong, Department of Obstetrics & Gynaecology, Hong Kong, China

Study question: Is there a difference in serum angiogenic profile on day LH+7 between pregnant and non-pregnant women undergoing frozen embryo transfer?
Summary answer: Serum PLGF level on day LH+7 in women who conceived was significantly lower than those who did not conceive.
What is known already: Angiogenesis is essential for successful pregnancy establishment. VEGF, PLGF and IGF1 are three main angiogenic factors which play significant role around the time of embryo implantation. Previous studies have shown that alternation in expression of angiogenic factors was associated with infertility and they may serve as predictive indicators for pregnancy outcome. However, the relationship between serum PLGF, VEGF and IGF1 and pregnancy outcome has not been fully illustrated.
Study design, size, duration: It is a prospective pilot study in a tertiary teaching hospital. A total of 40 infertile patients who underwent frozen embryo transfer treatment from Jan 2018 to Dec 2018 were recruited in our study.
Participants/materials, setting, methods: All the serum specimens were collected precisely on day 7 after LH surge in a natural non-conception cycle. Commercial Elisa kits were used to identify the concentrations of PLGF, VEGF, and IGF1. Demographic data and pregnancy outcomes were collected. Serum levels of PLGF, VEGF, and IGF1 were compared between patients who conceived (n=21) and who did not conceive subsequently (n=19).
Main results and the role of chance: There was no significant difference in maternal age BMI, transferred embryo numbers, endometrial thickness between the two groups. The serum concentration of PLGF in non-pregnant group (median 7.24, range 0-14.82pg/ml) was significantly (*P*=0.009) higher than it in the pregnant group (median 1.92, range 0-9.17pg/ml). IGF1 level was comparable between pregnant and non-pregnant patients (86.9±23.1 ng/ml vs. 87.2±26.5 ng/ml, *P*=0.971). There was no significant difference (*P*=0.088) in the serum VEGF level between non-pregnant group (median 233.3, range 78.89- 514.79pg/ml) and the pregnant group (median 139.0, range 50.47-379.34pg/ml).
Limitations, reasons for caution: The sample size is relatively small in this study and a large-scale study is needed to verify the results.
Wider implications of the findings: The altered expression of PLGF in serum may provide information on the prognostic value of serum angiogenic factor profile for pregnancy outcome.
Trial registration number: Nil

P-365 To compare effect of two protocol in hormone-replacement cycle for frozen-thawed embryo transfer - A single center, retrospective study

J. Gao[1], C. Ming-Hui[1], X. Yanwen[1], Z. Canquan[1]

[1]First Affilated Hospital Of Sun Yat-sen University, Reproductive Center, Guangzhou, China

Study question: What is the optimal dose for dydrogesterone combined used with progesterone vaginal gel for luteal-phase support(LPS) in hormone-replacement cycle for frozen-thawed embryo transfer.
Summary answer: 90 mg Crinone plus 20mg dydrogesterone BID has higher clinical pregnancy rate than 10mg dydrogesterone BID for LPS in hormone-replacement cycle for frozen-thawed embryo transfer.
What is known already: Vaginal progesterone gel (Crinone,8%) contains 90 mg of natural progesterone, which acts on the endometrium and myometrium and has some effect on inhibiting uterine contractions. The literature shows that the combination of Crinone and oral luteal-phase support drugs(dydrogesterone) can improve the pregnancy rate of hormone-replacement cycle for frozen-thawed embryo transfer. But what is the optimal dose for dydrogesterone is controversial problem.
Study design, size, duration: This retrospective study was conducted on 749 cycles who underwent hormone-replacement cycle for frozen-thawed embryo transfer from 1 Jan 2018 to 30 Nov 2019 at the First Affiliated Hospital of Sun Yat-sen University.
Participants/materials, setting, methods: 749 cycles underwent hormone-replacement cycle for frozen-thawed embryo transfer were enrolled. Patients used 4~9mg estradiol for 10~14 days in each treatment cycle. When the thickness of endometrium ≥8mm, progesterone was administered for 17 days. Among them, 427 cycles received 90 mg Crinone plus 10mg dydrogesterone BID(Group A), and 320 cycles received 90 mg Crinone plus 20mg dydrogesterone BID(Group B). Both of them receive single blastocyst embryo transfer at the sixth day of progesterone conversion.
Main results and the role of chance: There were no significant differences with regard to mean age, embryo transfer cycles, the thickness of endometrium on the day of progesterone conversion and embryo score between the two groups. The clinical pregnancy rate in group A was significant lower than group B (57.61% vs 66.56%, *P*=0.015).
Limitations, reasons for caution: This is a retrospective study. It suggest that 90 mg progesterone vaginal gel plus 20mg dydrogesterone BID may have better clinical pregnancy rate than 10mg dydrogesterone BID. Addition randomized prospective study are needed to confirm our finding, which is our future research direction.
Wider implications of the findings: We found that compare with 10mg dydrogesterone BID, 20mg dydrogesterone BID plus 90 mg progesterone vaginal gel would increase the clinical pregnancy rate in hormone-replacement cycle for frozen-thawed embryo transfer.
Trial registration number: no

P-366 The role of chromosomal aberrations of the embryo in the genesis of recurrent and sporadic miscarriage

E. Kudryavtseva[1], V. Kovalev[1], I. Baranov[2]

[1]Ural state medical university, Obstetrics and gynecology, Ekaterinburg, Russia C.I.S. ;
[2]National research center for obstetrics- gynecology and perinatology, organizational and methodological department, Moscow, Russia C.I.S.

Study question: Does the frequency and structure of chromosomal abnormalities in the embryo (fetus) differ with sporadic and recurrent pregnancy loss
Summary answer: Chromosomal abnormalities in the embryo are a significant cause of a miscarriage in both sporadic and recurrent
What is known already: About 50% of miscarriages are associated with the presence of chromosomal abnormalities in the embryo. It is assumed that if the patient suffers from recurrent miscarriage, that is, has a history of at least 2 consecutive cases of pregnancy loss, chromosomal abnormalities in the embryo are less common, and other reasons for the unsuccessful outcome of pregnancy should be sought. But in recent years there have been reports that, in a comparative analysis of the chromosome set in an embryo with sporadic and recurrent miscarriage, there was no significant difference in the frequency of occurrence of chromosomal pathology
Study design, size, duration: A retrospective cohort study was conducted by the method of continuous sampling, which included 1000 patients from Russia with a pregnancy demise diagnosed in the period of 6-12 weeks of gestation. The study was conducted in 2018-2019.
Participants/materials, setting, methods: The first group consisted of 681 patients whose first sporadic miscarriage was registered. The second group consisted of 319 patients who previously had a miscarriage. The products of conception (fresh samples of native biological material) obtained by vacuum aspiration were delivered to the laboratory where chromosomal microarray analysis was performed.
Main results and the role of chance: In group 1, various CAs in the embryo / fetus were detected in 378 samples (55.5%), in group 2 - in 203 samples (63.5%). The frequency of occurrence of chromosomal abnormalities in patients who already have a history of miscarriage was higher than with sporadic miscarriage - the differences are statistically significant (p = 0.015). There were no significant differences in the structure of chromosomal abnormalities. Autosome trisomy and numerical abnormalities of sex chromosomes were most often detected. So chromosomal abnormalities in the embryo are a significant cause of a miscarriage in both sporadic and recurrent. Structural rearrangements were detected in 52 samples (5.2%), and 30 (57.7%) of them were submicroscopic, and would not have been detected using standard cytogenetic studies. Genetic analysis of abortive material is an important component of the examination for the selection of further management tactics for patients. CMA is an effective research method when conducting genetic analysis of conception products.
Limitations, reasons for caution: In different ethnic groups, the frequency and structure of chromosomal abnormalities may vary
Wider implications of the findings: In many cases, a genetic study of abortive material makes it possible to clearly establish the cause of miscarriage, reduce the number of tests and diagnostic procedures assigned to patients, and optimize the algorithm of examination and preconception preparation.
Trial registration number: not applicable

P-367 Dydrogesterone addition to vaginal progesterone and transfer postponement improve outcome in patients with low P levels following hormonally substituted cycles for frozen-thawed embryo transfer

I. Cedrin - Durnerin[1], C. Vinolas[1], F. Krief[1], B. Dagher-Hayeck[1], M. Peigne[1], C. Sifer[2], S. Sarandi[2], B. Bennani Smires[2], M. Grynberg[1]

[1]Hôpital Jean Verdier, Service de médecine de la reproduction et préservation de la fertilité, Bondy, France ;
[2]Hôpital Jean Verdier, Laboratoire de biologie de la reproduction, Bondy, France

Study question: Does dydrogesterone addition to vaginal progesterone (P) and postponement of frozen thawed embryo transfer (FET) improve outcome in patients with low P levels following hormonal replacement therapy?
Summary answer: Dydrogesterone addition to vaginal P and 1-day FET postponement provide similar outcome in patients with low P levels compared to normal P "in phase" transfers.

What is known already: In artificial cycles for FET, low serum P levels on the day of transfer have been associated to decreased pregnancy and live birth rates. In our hands, measurement of serum P levels prior to transfer and if required, adjustment of vaginal P doses associated to 1-day FET postponement significantly improved outcomes. Despite this therapeutic strategy, more than 10% of cycles were cancelled for persistent inadequate serum P levels. Therefore, we modified our protocol by adding dydrogesterone to vaginal P in patients with low P levels prior to FET and similarly postponed embryo transfer by one day.
Study design, size, duration: This is a retrospective analysis of 249 prospectively collected FET cycles from November 2018 to October 2019. Endometrial preparation was achieved by sequential administration of vaginal estradiol until endometrial thickness >7 mm, followed by transdermal estradiol combined with 800 mg/day vaginal micronized P (400 mg twice a day). Our previous ROC analysis of serum P levels on FET day showed that optimal P level was 11 ng/mL to maximize sensitivity and specificity for live birth.
Participants/materials, setting, methods: This study was conducted in a university hospital. Serum P was measured on D1 following exogenous vaginal P introduction in the evening (referred as D0). When P levels were >11 ng/mL, embryo transfer was performed "in phase" on D2, D3 or D5 depending on embryo stage at cryopreservation. When P levels were <11 ng/mL, dydrogesterone (10 mg three times a day orally) was added to vaginal P and FET was postponed by one day.
Main results and the role of chance: Mean serum P level on D1 was 10.2 + 3.8 ng/mL, range (2.6-25.3 ng/mL). Serum P <11 ng/mL were observed in 63% of cycles (mean P level: 7.8 + 1.9 ng/mL vs. 14.1 + 2.9 ng/mL in patients with P >11 ng/mL, p<0.0001). On D1, serum estradiol levels were also significantly lower in patients with P <11 ng/mL (271 + 202 vs. 354 + 320 pg/mL, p<0.01). Therefore, 158 FET were performed with dydrogesterone addition from D1 onwards and postponed by one day while 91 FET were performed "in phase" following introduction of vaginal P. Characteristics of patients in both groups were similar for age (34 + 5 vs. 34 + 6 years), endometrial thickness prior to P introduction (9.6 + 2.0 vs. 9.7 + 2.2 mm), number of transferred embryos (1.4 + 0.5 vs. 1.5 + 0.5), embryo transfer stage (D2/D3/blastocyst: 7/32/61% vs. 5/38/57%). This strategy led to similar positive pregnancy test (37.3% vs. 40.6%, NS), heartbeat activity at 8 weeks (29.1% vs. 31.8%, NS) and ongoing pregnancy rates at 12 weeks (27.2% vs. 31.8 %, NS) between "dydrogesterone addition and 1-day postponement of FET" and embryo transfers performed "in phase".
Limitations, reasons for caution: The number of cycles has to be extended to confirm these preliminary data.
Wider implications of the findings: These results suggest that serum P measurement prior to ET followed by further addition of dydrogesterone to vaginal P and postponement of transfer might optimise the outcome of patients with low P levels in hormonally substituted FET cycles and avoid cancellation of a large number of cycles.
Trial registration number: not applicable

P-368 Endometrial thickness on the day of ovulation trigger is an effective predictor of pregnancy outcomes after frozen blastocyst transfer in spontaneous natural cycles

S. Onogi[1], K. Ezoe[2], S. Nishihara[1], J. Fukuda[1], T. Kobayashi[1], K. Kato[1]

[1]Kato Ladies Clinic, Gynaecology, Tokyo, Japan ;
[2]Kato Ladies Clinic, R&D division, Tokyo, Japan

Study question: Can the endometrial thickness (EMT) on the day of ovulation trigger predict the pregnancy outcomes after single vitrified-warmed blastocyst transfers (SVBTs) in spontaneous natural cycles?
Summary answer: The increased EMT on the day of the trigger was associated with improved ongoing pregnancy rate and decreased miscarriage rate in the first trimester.
What is known already: The relationship between the EMT on the day of embryo transfer (ET) and pregnancy outcomes has been controversial for

decades. Numerous studies reported that the decreased EMT on the day of ET was associated with a reduced likelihood of pregnancy; therefore, the EMT has been used as a predictor of pregnancy outcomes. However, recent studies demonstrated that the EMT on the day of ET is a poor predictor and has only a small independent prognostic value. The correlation of the EMT on the day of ovulation trigger with pregnancy outcomes after SVBT in spontaneous natural cycles is currently undetermined.

Study design, size, duration: A total of 901 SVBTs in spontaneous natural cycles, from November 2018 to October 2019, were analysed. Associations of EMT on the days of the trigger (EMT-Trigger) and SVBT (EMT-SVBT) with clinical and ongoing pregnancy rates were statistically evaluated. The factors possibly associated with EMT on the day of the trigger, such as patient and cycle characteristics, were also investigated.

Participants/materials, setting, methods: After monitoring follicular development and serum hormone levels, ovulation trigger was performed using a nasal spray containing buserelin, a gonadotropin-releasing hormone agonist. After ovulation was confirmed, SVBTs were performed on day 5. The EMT-Trigger and EMT-SVBT were evaluated using ultrasound on the day of the trigger and immediately before the SVBT procedure.

Main results and the role of chance: The patients were allocated according to the quartiles of EMT-Trigger as follows: EMT <8.1 mm, 8.1 mm ≤ EMT < 9.1 mm, 9.1 mm ≤ EMT < 10.6 mm, and EMT ≥10.6 mm. The increase in EMT-Trigger was significantly associated with the improvement of clinical and ongoing pregnancy rates ($P = 0.0003$ and $P < 0.0001$, respectively) and decreased miscarriage rates in the first trimester ($P = 0.0487$). The patients were also allocated according to the quartiles of EMT-SVBT: EMT <9.1 mm, 9.1 mm ≤ EMT < 10.1 mm, 10.1 mm ≤ EMT < 12.1 mm, and EMT ≥ 12.1 mm. EMT-SVBT was associated with clinical and ongoing pregnancy rates ($P = 0.0053$ and $P = 0.0010$, respectively), but not with the miscarriage rate ($P = 0.0848$). Additionally, multivariate logistic regression analysis demonstrated a significant correlation between the EMT-Trigger and ongoing pregnancy (adjusted odds ratio [AOR], 1.154; 95% confidential interval [CI], 1.046-1.274; $P = 0.0042$). However, no correlation was observed between EMT-SVBT and ongoing pregnancy (AOR, 1.043; 95% CI, 0.958-1.136; $P = 0.3251$). The decreased EMT-Trigger was significantly associated with an increase in female age ($P < 0.0001$) and shortened follicular/proliferation period ($P < 0.0001$).

Limitations, reasons for caution: The data used in this study were obtained from a single-centre cohort; therefore, multi-site studies will be required to ascertain whether these findings can be generalised to other clinics with different protocols and/or patient demographics.

Wider implications of the findings: This is the first report demonstrating a correlation between the EMT on the day of the trigger and pregnancy outcomes after frozen blastocyst transfer in spontaneous natural cycles. Our results suggest that EMT on the day of the trigger could be an effective predictor of pregnancy outcomes.

Trial registration number: not applicable

P-369 Impact of Preimplantation Genetic Testing for Aneuploidies (PGT-A) on first trimester biochemical markers - PAPP-A (placenta associated plasma protein) and free β-hCG (human chorionic gonadotropin)

D. Markova[1], K.O. Kagan[2], M. Hoopmann[2], H. Abele[2], E. Abecia[1], H. Fatemi[3], B. Lawrenz[3]

[1]IVI RMA Middle East Fertility Clinic, Fetal medicine, Abu Dhabi, United Arab Emirates ;

[2]Women's University Hospital- Tuebingen- Germany, Feto-Maternal Medicine, Tuibingen, Germany ;

[3]IVI RMA Middle East Fertility Clinic, Reproductive medicine, Abu Dhabi, United Arab Emirates

Study question: Is there a difference in concentrations of first trimester biochemical markers amongst patients with IVF/PGT-A, IVF/noPGT-A (fresh / frozen embryo transfer) and spontaneous conception?

Summary answer: There is a statistically significant difference in the concentrations of PAPP-A, but not in the levels of free β-hCG amongst the analysed groups.

What is known already: PAPP-A and free β-hCG are the two serum biomarkers used in calculation of the first trimester risk for chromosomal abnormalities and preeclampsia. It is a well-known fact that several factors like cigarette smoking, ethnicity and type of conception affect the levels of these two markers. The levels of PAPP-A are reduced and the levels of free β-hCG are increased in women who conceived through ART when compared with naturally conceived pregnancies. Moreover IVF/ICSI procedure, type of embryo transfer as well as freezing and thawing of embryos, might have an impact on the levels of first trimester biochemical markers.

Study design, size, duration: A cross-sectional, observational, collaborative, retrospective study was conducted between June 2009 and December 2019 in women with singleton pregnancies after either spontaneous conception, or IVF with PGT-A and frozen embryo transfer (FET) or after IVF without PGT-A and fresh transfer (ET) or FET and who had first trimester combined screening test between 10 and 14 weeks of gestation.

Participants/materials, setting, methods: In IVF/PGT-A-cycles, trophectoderm biopsy was performed. Serum PAPP-A and free β-hCG were measured at 10 to 14 weeks of gestation and analysed by Cobas E and Kryptor, certified by Fetal Medicine Foundation(FMF). Levels for both parameters were converted into Multiples of the Median (MoM) for corresponding gestational age by software of FMF. Both parameters were compared between the following groups: IVF/PGT-A/FET versus IVF/noPGT-A/FET; IVF/noPGT-A/ET versus IVF/noPGT-A/FET, spontaneous pregnancy versus IVF/PGT-A/FET or IVF/noPGT-A/ET and IVF/noPGT-A/FET.

Main results and the role of chance: The Kolmogorov-Smirnov test was used to analyse the normality of the distribution of variables. Difference of median of PAPP-A and free β-hCG were analysed by the Wilcoxon rank-sum test with nonparametric ANOVA. SAS studio™ software was used for statistical analysis.

A total number of 728 patients were included. Patients were divided into four groups; (i) IVF/PGT-A/FET (n=143); (iia) IVF/noPGT-A/ET (n=346) and (iib) IVF/noPGT-A/FET (n=100); (ii) naturally conceived pregnancies (n=139).

The median levels of PAPP-A were 0.97MoM; 1.12MoM; 1.20MoM and 1.03MoM respectively.

The median PAPP-A was the lowest in the group with IVF/PGT-A/FET and the highest in the group with IVF/noPGT-A/FET.

A statistically significant difference was observed in the median PAPP-A between IVF/PGT-A/FET group versus IVF/noPGT-A/FET group (p=0.01), spontaneous pregnancies versus IVF/noPGT-A/FET (p=0.01), and spontaneous pregnancies versus IVF/noPGT-A/ET (p=0.06).

No difference was observed when the group with naturally conceived pregnancies was compared with IVF/PGT-A/FET group (p=0.5) and in the group with IVF/noPGT-A/FET versus IVF/no PGT-A/ET (p=0.3).

The median levels of free b-HCG are 1.12MoM; 1.00MoM; 1.01MoM and 0.98MoM respectively.

Median free b-HCG was the lowest in the group with natural conception.

No statistically significant difference was observed in the concentrations of median level of free b-HCG amongst the compared groups (p>0.05).

Limitations, reasons for caution: The limitations of the study are the retrospective design and the ethnic diversity in the groups due to the multicentric study design.

Wider implications of the findings: PGT-A and ET-type have an implication on PAPP-A levels. The impact and pathophysiology of IVF related procedures (freezing and thawing of the embryo, trophoectoderm biopsy) on levels PAPP-A require further evaluation in order to assess the need for adjustments on the first trimester biochemical markers for pregnancies after IVF/PGT-A.

Trial registration number: Not applicable

P-370 Blastocyst morphology is not associated with maternal first trimester serum markers after fresh single embryo transfer (SET)

A. Pérennec[1], A. Reignier[1], T. Goronflot[2], P. Barrière[1], T. Fréour[1], T. Lefebvre[1]

[1]Department of Biology and Reproductive Medicine, Nantes University Hospital, Nantes, France ;

[2]Data Clinic- INSERM, Nantes University Hospital, Nantes, France

Study question: Is there an association between blastocyst morphology and maternal first trimester serum markers in In Vitro Fertilization pregnancies obtained after fresh single embryo transfer ?

Summary answer: Blastocyst morphology is not associated with serum levels of PAPP-A and b-hCG in first trimester screening after fresh single embryo transfer.

What is known already: Blastocele expansion, inner cell mass (ICM) and trophectoderm (TE) morphology are used to evaluate blastocyst implantation potential. Serum levels PAPP-A and b-hCG are used for first trimester combined screening, as they are associated with the risk of fetal aneuploidy and placental disorders. Several studies analyzed first trimester serum markers in IVF (In Vitro Fertilization) pregnancies and found lower PAPP-A level than in spontaneous pregnancies, while the results remained unclear regarding b-hCG levels. Several hypotheses have been raised to explain this apparent decrease, but the pathophysiology remains unclear, as well as the potential association between embryo morphology and early placentation.

Study design, size, duration: This bi-centric retrospective study was conducted between January 2012 and August 2018. We included 122 women aged from 18 to 43 years-old, whose pregnancy progressed at least beyond 13 weeks after a single blastocyst transfer and who participated in the first trimester combined screening test: 43 in a public IVF Center and 79 in a private IVF center.

Participants/materials, setting, methods: Day 5 and day 6 blastocysts were evaluated according to Gardner and Schoolcraft classification. Blastocysts were divided into three groups : excellent (≥ 3AA), good (3-6AB, 3-6BA, B2), medium to poor (3-6BB, 3-6AC, 3-6CA, B1, 3-6CB, 3-6BC). First trimester serum markers were measured in maternal blood between 11 and 13 weeks of amenorrhea + 6 days with Roche method and were expressed in MoM (multiple of median). Univariate and multivariate analyses were performed.

Main results and the role of chance: Body mass Index, smoking status, type of infertility, geographical origin, anti-mullerian hormone (AMH) level, ovarian stimulation characteristics, pregnancy outcomes and obstetrical complications were comparable between the three groups. Female age was statistically lower in the « medium to poor » group than in other groups. There were no significant differences in mean first trimester serum markers between the three groups (PAPP-A : p = 0,20 ;b-hCG : p = 0,12). No significant difference was found either between mean first trimester serum markers and ICM morphology (PAPP-A : p = 0,67 ; b-hCG : p = 0,60), TE morphology (PAPP-A : p = 0,66 ; b-HCG : p = 0,25), or blastocele expansion (PAPP-A : p = 0,22 ; b-hCG : p = 0,48). After adjustement on potential confounding factors (female age, type of gonadotropin, parity, number of oocytes retrieved and occurrence of ovarian hyperstimulation syndrom), the multivariate analyses did not report any significant association between PAPP-A or bHCG levels and blastocyst morphology.

Limitations, reasons for caution: "Poor" (≥B3CB and ≥B3BC) and "medium" (≥B3BB, ≥B3AC, ≥B3CA and B1) quality blastocysts were grouped together and represented only 27 blastocysts.

Wider implications of the findings: Our study concluded that first trimester serum markers were not statistically different according to blastocyst morphological characteristics. Although this needs further confirmation, this suggests that blastocyst morphology could have an impact on implantation, but not on placentation. Therefore, these findings are reassuring for couples undergoing IVF and blastocyst transfer.

Trial registration number: Not applicable

P-371 Endometrial fluid derived extracellular vesicles as low-invasive diagnostic biomarkers of implantative endometrium

J. Ibañez Perez[1], M. Diaz-Nuñez[2], L. Lainz[2], M. Diez-Zapirain[2], M. Iglesias[2], A. Expósito[2], M. Gonzalez[3], J.J. Lozano[4], A.M. Aranzay[3], E. Gonzalez[5], F. Royo[6], N. Subiran[7], R. Matorras[2], J.M. Falcon-Perez[8]

[1]CIC biogune- Cruces University Hospital, Human Reproduction Unit - Biocruces, Barakaldo, Spain ;
[2]Cruces University Hospital, Human Reproduction Unit - Biocruces, Barakaldo, Spain ;
[3]CIC bioGUNE, Genome Analysis Platform, Derio, Spain ;
[4]Bioinformatics Unit- Centre Esther Koplovitz CEK, CIBERehd, Barcelona, Spain ;
[5]CIC bioGUNE, Exosomes Laboratory, Derio, Spain ;
[6]Ciberehd- CIC bioGUNE-, Exosomes Laboratory-, Derio, Spain ;
[7]Department of Physiology- Faculty of Medicine and Dentistry- University of the Basque Country UPV/EHU, Biocruces, Leioa, Spain ;
[8]Ikerbasque- CIC bioGUNE, Exosomes Laboratory, Derio, Spain

Study question: Is it possible to define a simple, sensitive and reproducible low-invasive extracellular vesicles (EVs) -based method to allow the quick identification of an implantative endometrium?

Summary answer: It is possible to obtain and analyze EVs and EVs-associated miRNAs from a small volume of endometrial fluid samples.

What is known already: Increasing embryo implantation rates has become one of the greatest challenges in ARTs. Usually an endometrial-biopsy is done to identify a receptive endometrium, which prevents embryo transfer in the same cycle, as it is detrimental for the implantation. Implantation is a complex process, which requires a synchrony between the development of the embryo and the endometrium, but also, an adequate embryo-endometrial cross-talk. The presence of EVs as mediators of this communication has been describe in the endometrial fluid (EF). The molecular analysis of the content of the EVs from EF could be a non-invasive method to improve the implantation rates.

Study design, size, duration: The population under study consisted of a group of 45 women who assisted at the Human Reproduction Unit from January 2018 to June 2019.

The study was approved by our Institutional Ethical and Investigation Board (CEIC 09/54 and CEIC 11/45).

Participants/materials, setting, methods: The main inclusion criteria were; cycle duration between 27 and 29 days, normal uterine and ovarian ultrasound, no previous gynecological pathologies and age between 18 and 37 years.
A pool of EF was used for the establishment of a robust methodology for analyzing EVs from endometrial fluid in clinical settings, where the sample is limited and no sophisticated equipment is available. For that, five different methodologies were compared in triplicate.

Main results and the role of chance: From the five methods compared, two of them consisted in the direct extraction of RNA while in the other three; an enrichment of EVs was done before the RNA extraction. SmallRNAseq was performed to determinate the most efficient method and showed that the protocols with a previous enrichment step of EVs obtained a higher miRNA expression.

Once the best method was selected, it was applied in a set of real samples with different implantation outcome. The content of miRNAs (mainly associated with EVs) of endometrial fluid samples from women in whom the implantation was successful (n=15) and unsuccessful (n=15) were analyzed. The results obtained from the differential analysis of the set of samples with different implantation outcome are being analyzed and it is expected that the results will be available by the time this communication is presented.

Limitations, reasons for caution: The main problem when working with biological replicates is the impossibility to quantify the sample of origin before starting with the analysis. Therefore, finding an adequate way to normalize the samples is crucial for analyzing the results.

Wider implications of the findings: This work demonstrate that it is possible to obtain and analyze EVs and EVs-associated miRNAs from a small volume of endometrial fluid samples, which allows the use of EV-miRNAs as a non-invasive biomarkers for the detection of an implantative endometrium.

Trial registration number: not applicable

P-372 An increased % of CD56+CD16+ NK cells in the endometrial biopsy is not related with recurrent miscarriage when maternal Killer cell Immunoglobulin-like Receptors are considered

D. Alecsandru[1], A. Pacheco[2], N. Garrido[3], A. Barrio[4], P. Aparicio[5], A. Pellicer[6], J.A. Garcia -Velasco[4]

[1]IVIRMA MADRID, Department of Immunology- IVI RMA- Madrid- Spain, MADRID, Spain ;
[2]IVI RMA Madrid, Department of Andrology- IVI RMA- Madrid- Spain, Madrid, Spain ;
[3]IVI Foundation- Health Research Institute La Fe- Valencia- Spain., IVI Foundation-Health Research Institute La Fe- Valencia- Spain., Valencia, Spain ;
[4]IVI RMA Madrid, Department of Reproductive Endocrinology and Infertility- IVI RMA- Madrid- Spain, Madrid, Spain ;
[5]IVI RMA Madrid, Department of Immunology- IVI RMA Madrid- Spain, Madrid, Spain ;
[6]IVI RMA- Roma- Italy, Department of Reproductive Endocrinology and Infertility- IVI RMA- Roma- Italy, Roma, Italy

Study question: Could the increased % of CD56+CD16+ NK cells in the endometrial biopsy in oocyte donation recipients be considered as a risk factor for recurrent miscarriage?
Summary answer: An increased % of CD56+CD16+ NK cells in the endometrial biopsy is not related with recurrent miscarriage when maternal KIR genotype is considered
What is known already: There is still a current controversy regarding the relationship between an increased % of uNK cells in mid-secretory endometrium and its association with recurrent miscarriage. Increased risk of recurrent miscarriage (RM) has described in KIR AA mothers when the fetus has more HLA-C2 genes than the mother, and this HLA-C2 are paternally or egg donor inherited. In ART oocyte donor cycles, oocyte HLA-C behaves as the paternal HLA-C and KIR-HLA-C combination is not currently taken into consideration on donors' selection. KIRAA women have lower live birth rates (LBR) after double embryo transfer (DET).
Study design, size, duration: Between January 2017 and June 2019 we performed a prospective study of 139 women undergoing one cycle of oocyte donation-ART. They had recurrent miscarriage (RM) of unknown etiology. Thirty nine out of 139 patients underwent an endometrial biopsy under sterile conditions with a Pipelle catheter in the month prior of the embryo transfer (ET). Twenty-one healthy oocyte donors were included as a control group and they had an endometrial biopsies too.
Participants/materials, setting, methods: All the patients were selected from IVI RMA Clinics. KIR and HLA-C typing was performed for patients and HLA-C for partners and oocyte donors. They had a normal thrombophilia tests. The miscarriage and LBR has analyzed. The immune cell populations on biopsy were analyzed by 3 techniques: flow cytometry, immunohistochemistry and gene expression. A HLA-C tetramer has used to investigate the mother KIR biding to the embryo HLA-C.
Main results and the role of chance: The median age of our patients was 40 years, and 25 years for oocyte donors. In our cohort the pregnancy rate was 71.2%, the miscarriage rate was 31.6% (20.8% were clinical miscarriages), and the live birth rate was 39.5%. A higher miscarriage rate after DET in KIR AA (47.8%) compared to KIR AB (10.5%) and KIR BB (6.7%) ($p < 0.003$) was observed. A significantly decreased LBR has observed after DET in KIR AA (4.3%) compared to AB (26.3%) or BB (46.7%) ($p=0.009$). We analyzed the % and gene expression of CD56bright, CD56+CD16+, TregCD25+CD4+FoxP3+ cells. No differences were observed on CD56bright or Tregs when compared to controls. A higher CD56+CD16+ % was observed in patients (median 10.2), when compared to controls (6.3) $p<0.01$, but no differences were observed by KIR genotype among patients.

A lower gene expression (1/FC) of IL-10 was observed in the patients group compared with the control group ($p<0.02$), but no differences were observed by KIR genotype among patients. No differences have observed on TNFalpha gene expression between patients and control nor either by KIR genotype among patients.

There weren´t any differences on HLA-C tetramer binding to the uNK cells between groups or by maternal KIRs.
Limitations, reasons for caution: Our sample was small and this is the first report analyzing the reproductive outcome in oocyte donation by the endometrial % of CD56+CD16+ NK cells and maternal KIR. However, apart from statistical significance, the association strength was noticeably high, which confers our findings more confidence.
Wider implications of the findings: A higher miscarriage rate was observed in KIR AA patients but there weren´t differences in the % of endometrial CD56+CD16+ NK cells between KIR AA, AB or BB. Their increased % observed in the patients when compared to control group cannot be related with an increased risk of miscarriage.
Trial registration number: Not applicable

P-373 Efficacy of Endometrial Scratching on Pregnancy rate in women with Unexplained and Mild Male Factor Infertility after Failed Intrauterine Insemination Cycles A Randomised Controlled Cycle

C. Thyagaraju[1], M.S. Madhuri[1], P. Dasari[1]

[1]*JIPMER, Obstetrics and Gynecology, Pondicherry, India*

Study question: Does Endometrial Scratching(ES) in proliferative phase increase the probability of pregnancy in women undergoing Ovulation Induction and Intrauterine Insemination(IUI) after previous failed IUI cycles?
Summary answer: Endometrial Scratching in stimulated IUI cycle is a cost-effective and easy technique which may improve clinical pregnancy rate in women with previous failed IUI cycles
What is known already: Endometrial Scratching(ES) has been suggested as an intervention to increase the probability of pregnancy in women undergoing IVF. Majority of studies reported that ES improves outcome in IVF, IUI and natural conceptions: however, the size and quality of studies are poor which really questions the presence of any beneficial effect. Endometrial injury is often performed by pipelle biopsy that has an established safety profile. However, it is also known to be associated with a moderate degree of discomfort/pain and bleeding and requires an additional pelvic examination. Neither of these studies reported the pain/discomfort and bleeding secondary to the procedure.
Study design, size, duration: A prospective randomized controlled trial. One hundred sixty-two women (81 in each group) with primary/secondary infertility were randomized into intervention and control group. Sample size was estimated using the statistical formula for comparing two proportions. The minimum expected difference in the pregnancy rate between the group is considered as 20% and the sample size is estimated at 5% level of confidence and 80% power. The study was conducted from June 2017 to June 2019
Participants/materials, setting, methods: Couples aged between 25 to 35years attending the Infertility outpatient clinic in OBG department, JIPMER, Pondicherry, India. Unexplained or mild male factor infertility with previous up to 3 failed IUIs were randomised according to random blocks. Three cycles of ovulation induction with Clomiphene citrate and Gonadotrophins followed by IUI was done. ES was done using pipelle on day8/ 9 of the stimulated cycle in the intervention group. Pain and bleeding were noted after the procedure.
Main results and the role of chance: The mean age of women was 29.1 ± 3.4 years. There was a significant difference in the mean duration of infertility between two groups with a P-value of 0.041. There was no statistical difference in the distribution of type of infertility or unexplained and mild male factor infertility ($p=0.17$, $p=0.807$ respectively). There was no statistically significant difference with respect to the number of prior failed IUI ($p=0.279$). The clinical pregnancy rate in the ES group was 22.2% in comparison 9.8% in the control group. In the intent to treat analysis, with a P-value of 0.03 calculated from Chi-square test($p<0.05$) there was statistically significant difference in the pregnancy rate between Intervention and Control group. There is less than a 3% chance that the differences are not real. Efficacy of intervention was found to be Fourteen Percent (14%). Two patients in ES group had abortions and either of the group had no multiple pregnancies. There was an increased pregnancy rate (38.8%) in the third IUI cycle in the Intervention group compared to the Control group (12.5%)($p=0.43$). Fifty-one women (63%) had marked a VAS pain score of 4-5 with Mean VAS score of 3.42 ± 1.35. Twelve women (12.2%) experienced mild spotting post-procedure.
Limitations, reasons for caution: Small Sample size. Older age group might have led to a low pregnancy rate. Many unknown factors besides poor endometrial receptivity might have led to repeated failure cycles and reduced CPR. ES was done using the gentle movement of Pipelle which might have led to less inflammatory response for implantation.
Wider implications of the findings: Endometrial Scratching in IUI will be an inexpensive alternative to IVF for couples after IUI failures especially in developing countries, with an acceptable pregnancy rate and does not demand any special qualification. Larger and adequately powered studies are needed to elucidate the beneficial effects of endometrial scratching on Implantation.
Trial registration number: Trial REF/2017/10/015540

P-374 When is a pregnancy of unknown location considered to be non-viable and ready to treat?

K. Barnhart[1], B. Nolan[2], T. Thomas[3], E. Steider[4], W. Youssef[5], K. Hansen[6], A. Steiner[7], M. Stephenson[8], M. Alston[9], M. Cedars[10], E. Jungheim[11], M. Diamond[12], H. Zhang[13]

[1]*University of Pennsylvania Medical Centre, Dept of Ob/Gyn, Philadelphia Pennsylvania, U.S.A. ;*
[2]*University of Pennsylvania, Department of Ob/Gyn, Philadepphia, U.S.A. ;*
[3]*Univiersity of Pennsylvania, Dept of Ob/Gyn, Philadelphia, U.S.A. ;*
[4]*University of Pennsylvania, Dept of Ob/Gyn, Philadpephia, U.S.A. ;*
[5]*University of Pennsylvania, Dept of Ob/Gyn, Philadelphia, U.S.A. ;*
[6]*University of Oklahoma, Dept of Ob/Gyn, Oklahoma City, U.S.A. ;*

[7]Duke University, Dept of Ob/Gyn, Durham, U.S.A. ;
[8]University of Illinois at Chicago, Dept of Ob/Gyn, Chicago, U.S.A. ;
[9]Denver Health, Dept of Ob/Gyn, Denver, U.S.A. ;
[10]University of California San Francisco, Dept of Ob/Gyn, San Francisco, U.S.A. ;
[11]Northwestern University, Dept of Ob/Gyn, Chicago, U.S.A. ;
[12]Augusta University, Dept of Ob/Gyn, Augusta, U.S.A. ;
[13]Yale University, Dept Biostatistics, New Haven, U.S.A.

Study question: Are criteria that determine when an early pregnancy is nonviable conservative enough to enroll women in a clinical trial regarding treatment?
Summary answer: Conservative criteria adopted to diagnose a nonviable pregnancy of unknown location as part of a RCT did not eliminate enrollment of a viable gestation.
What is known already: Current clinical criteria to consider a pregnancy non-viable vary in clinical guidelines. When an early gestation is not visualized with ultrasound, serial hCG values are used to determine viability prior to potential intervention. NICE 2019 defines a change of 63% or greater over 48 hours as potentially viable. ACOG 2018 defines a minimal rate consistent with a viable gestation to be 49% or greater over 48 hours (for initial values under 1500 mIU/mL). A viable intrauterine gestation has been noted with a rate of as low as 35% over 48 hours (Seeber et al. Fert and Sert,2006;86:454-459.).
Study design, size, duration: ACT or NOT is an RCT that evaluated the treatment of women with a persistent pregnancy of unknown location. Inclusion criteria included no definitive ultrasound evidence of intrauterine or extrauterine gestation and a plateau in hCG, defined as a < 30% in 2 days, < 50% in 3 days, < 75% at 4 days, < 100% at 5 days, < 130% at 6 days or >166% at 7 days. Two clinicians confirmed eligibility.
Participants/materials, setting, methods: After determination of eligibility subjects were randomized 1:1:1 to expectant management and to two different active treatment arms.
Main results and the role of chance: A participant was enrolled, randomized to expectant management, and was later noted to have a viable gestation. A 33-year-old, G2 P1001, who conceived with use of clomiphene citrate and IUI was enrolled at 4 5/7 weeks gestation. She presented with abnormal serial hCG values of 7% in 2 days and 24% over 4 days: 86 at GA 4 0/7, 92 at 4 2/7 and 107 at 4 5/7. Ultrasound at GA of 4 5/7 demonstrated no evidence of an intrauterine or extrauterine gestation. Subsequently hCG values rose normally: 348 at 5 0/7, 803 at 5 2/7, and 2477 at 6 0/7. A viable IUP was diagnosed at 6 5/7 with a CRL 7.7mm and cardiac activity. She elected to proceed with prenatal care and delivered at term without complication. This event was reported to the DSMB and IRB and judged to be unanticipated based on current clinical standards. However, a memo was circulated to all participating centers to use caution against declaring non-viability when; a) using early hCG values prior to 6 wks gestation when the date of conception is known, b) the hCG is continuously rising, and c) in those who may be at greater risk for multiple pregnancy.
Limitations, reasons for caution: While this case represents a single event from a larger RCT, it highlights the limitations of clinical guidelines based solely on laboratory tests. Criteria to classify an early gestation as non-viable, based on only hCG values, may not be 100% predictive and should include all aspects of the clinical setting.
Wider implications of the findings: Despite conservative criteria, a viable gestation was unintentionally enrolled in trial designed to manage a non-viable gestation. If this participant was actively treated her pregnancy may have been interrupted. Current clinical standards may need to be amended to ensure a pregnancy of unknown location is nonviable prior to treatment.
Trial registration number: NCT01800162

P-375 Predictive value of serum HCG concentrations in pregnancies achieved after thawing embryo transfer

S.J. Chon[1]

[1]Gil Hospital, Obstetrics and Gynecology, Incheon-si, Korea- South

Study question: What would be cut off values of serum HCG level in maternal blood measured on seventh day of thawing blastocyst transfer to confirm stable clinical pregnancy and live birth rates?
Summary answer: The cut off values predicting a clinical pregnancy was 22.8 IU/L and for ongoing pregnancy was 26.45 IU/L.
What is known already: Threshold values for predicting a clinical pregnancy for a fresh blastocyst were 111IU/l and for a vitrified-warmed blastocyst 137IU/l.
Study design, size, duration: A cryopreserved thawing embryo transfer (T-ET) cycles were retrospectively analyzed a database of clinical and laboratory information between 1 January 2015 and 30 February 2018 at a single fertility center.
Participants/materials, setting, methods: In the present study, a total of 166 patients with T-ET were included and only serum HCG levels drawn 7 days after the T-ET were included. The percentage for the area under the curve and the 95% confidence interval were generated for each ROC curve. The AUC measures the diagnostic accuracy of HCG on pregnancy outcome; an AUC closer to 1.0 denotes a perfect test.
Main results and the role of chance: Among 166 patients who underwent T-ET, 89 resulted in a positive maternal serum HCG; 31 resulted in a biochemical pregnancy, and 14 in clinical abortion, and 44 patients achieved an ongoing pregnancy and 43 of them finally achieved live births. Of the 44 ongoing pregnancies, 43 live births were successful in the T-ET cycle of which difference were very small, however they were shown separately because of the significant meaning of live births. The cut off values predicting a clinical pregnancy was 22.8 IU/L with sensitivity of 91.4% and a specificity of 91.7%. The cut off values predicting an ongoing pregnancy for a T-ET was 26.45 IU/L, with a sensitivity of 93.2%, a specificity of 84.4%, and live birth was 27.95 IU/L, with a sensitivity of 90.7 %, a specificity of 84.6 %.
Limitations, reasons for caution: This is a retrospective study with small sample size and it is a single centered study.
Wider implications of the findings: Clinicians could inform patients who have undergone thawing single embryo(blastocyst) transfer whether their positive serum hCG would present better outcomes or not based on these cut off values.
Trial registration number: GBIRB2019-310

P-376 4D ultrasonographic evaluation of uterine peristalsis correlates with progesterone levels in patients with repetitive implantation failure.

B. Moliner, M.D.[1], **J. Llacer**[1], **A. Bernabeu**[1], **J.C. Castillo**[1], **A. Fuentes**[1], **F. Sellers**[1], **R. Bernabeu**[1]

[1]Instituto Bernabeu, Reproductive Medicine Department, Alicante, Spain

Study question: Does 4D ultrasonographic evaluation of uterine peristalsis on the day of embryo transfer correlate with progesterone levels in patients with repetitive implantation failure (RIF)?
Summary answer: Uterine peristalsis evaluated by 4D ultrasound shows correlation with progesterone levels when assessed on the day of embryo transfer in patients with RIF.
What is known already: The role of progesterone in the inhibition of uterine peristalsis is widely known. Uterine peristalsis and progesterone levels on the day of embryo transfer are described as potential factors influencing pregnancy outcomes in IVF cycles. However, reliable methods demonstrating a correlation between these two entities specifically in patients with RIF are still under research.
Study design, size, duration: Our retrospective cohort study (November 2017-January 2020) included all consecutive IVF cycles of RIF patients (n=153) who underwent uterine contractility/progesterone levels assessment on the day of embryo transfer. RIF was defined as an unsuccessful implantation of a total number of ≥3 blastocysts originating from oocyte donation or autologous PGT-A cycles. Participants underwent frozen embryo transfer cycles (FET) and were evaluated for uterine contractions and serum progesterone levels 30 minutes before their embryo transfer procedure.
Participants/materials, setting, methods: Uterine contractions (UC) were assessed by recording a 6-minute-long video using 2D vs 4D mode (Voluson E10) by a single operator (BM). Contractions were counted visually on a 15x accelerated recording. In order to define low progesterone levels, we separated progesterone levels in quartiles, counting last quartile as Low progesterone level group (LP=< 9,2 ng/ml; N=38), considering the rest as Normal Progesterone level (NP>9,2 ng/ml; N=115).
Main results and the role of chance: Overall, patients mean age was 41 years, with an average of 4.1 embryo transfers performed previously and with

an average of 5.1 embryos transferred. The average of contractions measured in 2D was 0.62 contractions per minute (cpm) while the average of contractions measured in 4D was 0.99 cpm. There were no differences between the groups of uterine factor (adenomyosis, myomas, adhesions or polyp) or endometriosis, as well as of the variables previously mentioned such as age, the number of embryo transfers made previously or the number of embryos transferred previously. Differences were observed in uterine contractility measured by 4D ultrasound, observing 1.3 cpm in LP group while in HP group an average of 0.9 cpm was observed. This difference was statistically significant (p = 0.012) giving a RR of 1.6 (95% CI 0.6 to 4). However, uterine contractility assessment using 2D didn't show statistically significant differences (p = 0.315), observing a mean of 0.8 cpm in the LP group and a mean of 0.6 cpm in the NP group.

Limitations, reasons for caution: The inherent risk of bias associated to retrospective studies. Additionally, the small sample size -especially for the subgroup analysis (normal vs low progesterone levels)- precludes robust conclusions. Caution is warranted in extrapolating these results to patients without previous implantation failure.

Wider implications of the findings: Our findings suggest that low progesterone levels constitute a significant factor for increased uterine contractility. Furthermore, 4D ultrasound appears to be a reliable method for assessment of uterine peristalsis, thus should be considered for routine evaluation in RIF patients undergoing cryotransfer in artificial cycles.

Trial registration number: None

P-377 Anti-Müllerian hormone receptor type II causing repeated implantation failures through the endometrium apoptosis of the luteal phase

F. Yunxing[1], H. Rong[2]

[1]18309609178, Ningxia Medical University Shuang Yi Campus, Yinchuan, China ; [2]Ningxia Medical University / Ningxia Medical University General Hospital, Reproductive Medicine Center of Ningxia Medical University General Hospital, Yinchuan, China

Study question: To detect the expression of AMH and anti-Müllerian hormone receptor (AMHR)II at the maternal–fetal interface and explore whether AMH and AMHRII participated in the occurrence of repeated implantation failure (RIF).

Summary answer: AMHRII had an effect on RIF via the AMH/AMHRII signaling pathway. It participated in the occurrence of RIF by interfering with endometrial decidualization and apoptosis.

What is known already: The abnormal expression of key proteins and hormones at the maternal–fetal interface affected the maternal–fetal communication and led to adverse pregnancy outcomes. The expression of AMHRII in endometrial tissues was closely related to the reproductive ability of mice.

Study design, size, duration: From January 2015 to May 2016. The 154 endometrial tissues in the early follicular, follicular, and luteal phases were collected from 41 women with infertility due to tubal or male factors alone; 41 samples were also collected from patients with RIF. The 20 placental tissues were collected in early, mid and late pregnancy respectively. 12 blastocysts were cultured from nontransplanted fourth-order embryos with poor quality on the third day.All patients age were less than 35.

Participants/materials, setting, methods: 1. Relative expression of AMH and AMHRII in Endometrium and placenta were analyzed using immunohistochemistry,WB and qRT-PCR. 2. Expressions of AMH and AMHRII mRNA in blastocysts detected using a single-cell real-time polymerase chain reaction. 3. Apoptosis in the endometrium during the luteal phase detected by TUNEL and WB. 4. The expression of estrogen receptor (ER), IGFBP1, and PRL were tested via immunohistochemistry. 5. The relationship between AMHRII and ER, IGFBP1, and PRL.

Main results and the role of chance: AMH and AMHRII were less expressed in the normal endometrium during the menstrual cycle. AMHRII was slightly highly expressed in the endometrium during the menstrual cycle, especially in the luteal phase. AMHRII was highly expressed in the placental tissues during early pregnancy and in the second and third trimesters. In addition, the expression of AMHRII mRNA was detected in the blastocysts, but no expression of AMH mRNA was observed. Positive expression of AMHRII in patients with RIF was 29.3%, which was higher than that in patients of normal childbearing age, especially during the luteal phase. TUNNEL and WB results indicated the proportion of patients with RIF and high expression of AMHRII was significantly higher than the proportion of patients with RIF and low expression of AMHRII and the proportion of normal individuals.The ER, IGFBP1, and PRL expression in the endometrium of patients with RIF and high expression of AMHRII was significantly lower than that in patients with RIF and normal expression of AMHRII. A negative correlation was found between the expression of AMHRII and ER, IGFBP1 and PRL in the endometrium during the luteal phase in patients.

Limitations, reasons for caution: Clinical samples are more difficult to obtain.Further studies with more samples are required to tested. Interfering gene expression at the cellular level to elucidate the exact underlying molecular mechanisms about AMHRII in RIF.

Wider implications of the findings: This study was novel in reporting the expression of AMH and AMHRII at the normal maternal–fetal interface. The abnormally increased expression of AMHRII might participate in the occurrence of RIF via a mechanism associated with the interference of endometrial decidualization and apoptosis, ultimately resulting in impaired endometrial receptivity.

Trial registration number: not applicable

P-378 Human endometrial glandular organoids: a powerful 3D-cell model to mimic morphological and molecular changes of the receptive endometrium at the implantation window

A. Luddi[1], V. Pavone[1], G. Morgante[1], L. Governini[1], B. Semplici[1], L. Benincasa[1], V. De Leo[1], P. Piomboni[1]

[1]University of Siena, Molecular and Developmental Medicine, Siena, Italy

Study question: Does a 3D-culture model of human endometrial glandular organoids mimic the morphology and the gene expression profile of a receptive endometrium at the implantation window?

Summary answer: Endometrial organoids are surrogate as regard morphology, creation efficiency, expandability, gene expression profile; pinopodes, reliable marker of endometrial receptivity, are detectable after *in vitro* decidualization.

What is known already: Many evidences support the existence in the human of a narrow window of uterine receptivity, which opens during the mid-luteal phase, during which endometrium becomes receptive toward the embryo. At this time, formation of pinopodes on the apical membranes of the endometrial epithelial cells occurs, a phenomenon that is considered as a reliable morphologic marker of endometrial receptivity. Due to ethical concerns to study human implantation *in vivo,* this *in vitro* three-dimensional culture system offers the opportunity to evaluate the endometrial microenvironment and to investigate the molecular mechanisms underlying the implantation process.

Study design, size, duration: Endometrial biopsies have been collected from healthy women, who underwent ovarian surgery from January to October 2019. The epithelial cells were isolated and 3D culture was applied to obtain organoids. Morphology, ultrastructure as well as gene expression profile of key genes known to be involved in the implantation process have been evaluated, before and after in vitro hormonal treatments to induce morphological and molecular modifications mimicking the proliferative and secretive phase of the uterine cycle.

Participants/materials, setting, methods: Endometrial specimens have been collected from women of proven fertility (n=5; 22-39 years old). Organoids of epithelial cells have been obtained, and then exposed for 4 days to estrogen alone or in combination with progesterone and cAMP, mimicking the proliferative and the secretory phase, respectively. Then, organoids were fixed and processed for TEM and SEM analysis or processed for RNA extraction and gene expression analysis by digital droplet PCR (ddPCR).

Main results and the role of chance: Our data highlight that organoids obtained from human endometrium represent a good model to study the physiological function of the endometrial epithelium. TEM analysis showed that organoids preserve glandular organization as well as ultrastructural characteristics. Moreover, a scanning electron microscopic (SEM) survey of endometrial organoids, definitively demonstrates the dramatic changes occurring in the luminal surface according to hormonal treatment. In organoids treated with E2, mimicking the proliferative phase, the luminal surface is relatively smooth or exhibit irregular micro-extensions; indeed, it appears overlaid with a rich net of microvilli and cilia clusters of different length. The organoids mimicking the secretory phase are characterized by the presence of large cytoplasmic apical protrusions traditionally called "pinopodes", that bulge out of the entire luminal cell surface. These protrusions have regular contours and are characterized by a

significant loss of free surface micro-extensions. These data are also confirmed by ddPCR analysis, that revealed significant modulation of genes involved in the endometrial receptivity depending on hormone treatment: when 3D-cultured organoids were supplemented with E2, the expression of *PGR*, *MMP26* and *PAEP* significantly increased if compared to control. More interestingly, E2+MPA+cAMP treatment increased the expression of HOXA 10, *IGF1*, *VEGF*, *MMP26* and *PAEP*, while *ESR1* significantly decreased.

Limitations, reasons for caution: Larger study needs in order to confirm these data, above all for supporting conclusions from gene expression profiles. Moreover, further development of this approach based on the incorporation of other endometrial cell types is looked-for a better understanding of mechanisms underlining the embryo implantation establishment.

Wider implications of the findings: 3D-culture model of glandular endometrium not only represents a valuable research tool to study molecular mechanisms underlying human implantation *in vitro*, but also will provide a valuable model to carry out comparative studies on specific mechanisms that may drive endometrial dysfunction, paving the way to the setup of personalized treatments.

Trial registration number: Not applicable

P-379 management and outcome of interstitial pregnancy after in vitro fertilization/embryo transfer: a retrospective consecutive series of 185 cases

C. Wang[1], S. Yang[1], R. Li[1], J. Qiao[1]

[1]Peking University Third Hospital, Obstetrics and Gynecology, Beijing, China

Study question: Surgical management of interstitial pregnancy (IP) with or without intrauterine pregnancy (IUP): cornual resection or cornuostomy?

Summary answer: Compared with cornual resection, cornuostomy by laparoscopy showed no difference in the removal of ectopic pregnancy and the preservation of intrauterine pregnancy.

What is known already: IP is a rare type of ectopic pregnancy, but its incidence rises among patients undergoing in Vitro Fertilization/Embryo Transfer (IVF/ET) treatment. Most patients show no specific clinical manifestations, but once the interstitial portion ruptured, patients may face serious life threats.

Study design, size, duration: A total of 185 cases who were diagnosed with IP followed IVF/ET and treated in our hospital between Jan 2010 and Oct 2019 were included, of which 66 (35.68%) were interstitial heterotopic pregnancy (IHP). The data of each patient, including age, history of gynecologic surgeries, details of IVT/ET process, therapeutic interventions and reproductive outcome, were collected through electronic medical record database and analyzed retrospectively.

Participants/materials, setting, methods: Measurement data were presented as mean±SD or median (minimum-maximum), and statistical significance of differences among the groups was analyzed by Student's t-test or Mann–Whitney U test. Categorical variables were reported as n%, and statistical significance of differences among the groups was analyzed by chi-square test or Fisher's exact test. A two-sided p value< 0.05 was considered statistically significant. All statistical analyses were performed with the use of SPSS statistics for Windows version 22.0 software.

Main results and the role of chance: The average number of embryos transferred was 2.08±0.416. 171 patients received Day 3 embryos and 14 patients received Day 5 blastocysts. 118 patients underwent fresh ET and 67 patients underwent frozen ET. There were no differences between IP without IUP patients and IHP patients on number of embryos transferred (2.034±0.43 vs 2.136±0.35, p=0.098) or type of ET (fresh ET 68.91% vs 54.55%, p=0.052), but IP without IUP patients received more Day 3 embryos transferred (94.96% vs 86.36%, p=0.040), and showed earlier gestational age (days) at diagnosis (43.84±6.08 vs 50.68±10.16, p<0.001). Patients are treated mainly by surgery, including 9 laparotomies, 113 laparoscopic cornuostomies (LC), 30 laparoscopic cornual resections (LCR) and 7 laparoscopic loop ligatures. LC and LCR shared the similar duration of surgery (minutes) [51 (9-189) vs 61(23-155), p=0.317] and intraoperative blood loss [5 (1-300) vs 10 (2-200), p=0.317]. There were no significant differences between LC and LCR on live birth rate (91.4% vs 77.8%, p=0.574) among IHP patients and persistent ectopic pregnancy rate (6.3% vs 0.0%, p=0.560) among IP without IUP patients. No surgical complications (including infection, uncontrolled bleeding, uterine rupture during subsequent pregnancy or delivery) were observed in our cohort.

Limitations, reasons for caution: This is a retrospective case-control study, which theoretically has selection bias, information bias and confounding bias. Although it has been controlled in the design and implementation, relevant bias interference cannot be completely excluded. In addition, the sample size is relatively small, although literature with larger samples never have been published.

Wider implications of the findings: Surgical management is a safe and effective approach for IP and even for IHP. Compared with cornual resection, cornuostomy by laparoscopy showed no difference in the removal of ectopic pregnancy and the preservation of intrauterine pregnancy, but the risk of persistent ectopic pregnancy was worthy of attention.

Trial registration number: Not applicable

P-380 Female lifestyle factors and risk of recurrent pregnancy loss: systematic review and meta-analysis

K.Y.B. Ng[1,2], G. Cherian[1], A. Kermack[1,2], S. Bailey[1,2], N. Macklon[3,4], S. Sunkara[5], Y. Cheong[1,2]

[1]Princess Anne Hospital, Obstetrics and Gynaecology, Southampton, United Kingdom ;
[2]University of Southampton, School of Human Development and Health, Southampton, United Kingdom ;
[3]University of Copenhagen, Zealand University Hospital, Copenhagen, Denmark ;
[4]London Women's Clinic, London Women's Clinic, London, United Kingdom ;
[5]Kings College London, Department of Women and Children's Health, London, United Kingdom

Study question: Do female lifestyle factors influence the risk of recurrent pregnancy loss (RPL)?

Summary answer: Overweight and obese women had a significantly higher risk of RPL; the impact of smoking, alcohol consumption and caffeine intake on RPL remains uncertain.

What is known already: Recurrent pregnancy loss, defined as the occurrence of two or more consecutive miscarriages occurs in 1-2% of couples, and is a diagnosis that remains unexplained in a large proportion of cases. Whilst the specific mechanisms leading to RPL are still relatively unknown, poor lifestyle is associated with a hostile reproductive environment, which may compromise optimal embryo implantation and securement of a pregnancy. Although associations between certain lifestyle factors and the risk of sporadic miscarriage have been reported, whether lifestyle factors impact the risk RPL is less well known. It is important to understand any probable associations to improve patient management.

Study design, size, duration: This is a systematic review and meta-analysis addressing the impact of female lifestyle factors on the risk of RPL. Electronic databases including Medline, Embase, Cochrane Library, CINHL and Pubmed were searched until August 2019. The lifestyle search terms included 'diet', 'smoking', 'alcohol', 'caffeine', 'exercise' and 'BMI'. The search term for miscarriage history included 'miscarriage', 'recurrent pregnancy loss', 'recurrent miscarriage' and 'spontaneous abortion'.

Participants/materials, setting, methods: Full text manuscripts were reviewed for relevancy by two independent authors. Studies were included if they explored women of reproductive age, who had been exposed to an aspect of female lifestyle, such as obesity, being overweight, underweight, smoking and alcohol intake. The outcome assessed was the risk of having RPL in this population. Where possible, individual study estimates were pooled using either fixed or random effects meta-analysis.

Main results and the role of chance: A total of 24,705 records were identified from the electronic search and an additional 192 records identified through hand searching of references. Fifteen studies were included in the systematic review and meta-analysis; 8 case control studies, 4 cohort studies, 2 survey based studies and 1 observational study. Meta-analyses of 4 studies showed that the odds of RPL in the obese compared to women with normal BMI is 1.79 (95% CI 1.34, 2.41). The odds of RPL in women with BMI>25 compared to women with normal BMI is also significantly increased (OR 1.33, 95% CI 1.13, 1.56). Meta-analysis of 5 studies showed that being underweight does not increase the risk of RPL (OR 1.00, 95% CI 0.59, 1.70). Meta-analysis of 3 studies shows no increase in risk of RPL in women who smoked cigarettes compared to non smokers (OR 1.62, 95% CI 0.90, 2.93), and no increased risk of RPL in women who consumed alcohol compared to those who do not (OR 1.12, 95% CI 0.88, 1.44). Meta-analyses of 2 studies showed no increased risk

of RPL in women who have higher caffeine intake (>99mg/day) than women who have lower caffeine intake (≤99mg/day) OR 1.35 (95% CI 0.97, 1.89).
Limitations, reasons for caution: This review is limited by heterogeneity in the definition of RPL and methods of quantifying smoking, alcohol and caffeine intake between studies. Some studies did not restrict on whether the miscarriages were consecutive. This review was limited to studies in English and we did not assess impact of male lifestyle.
Wider implications of the findings: Being overweight and obese contributed significantly to increased risk of RPL by over 1.3- and 1.8-fold respectively. Whether lifestyle interventions including weight loss programmes are beneficial require further investigation. Smoking, alcohol and high caffeine intake did not increase the risk of RPL, but evidence is limited given very few studies.
Trial registration number: not applicable

P-381 Regulation of the immune tolerance-inducing Human Leukocyte Antigen-G (HLA-G) for induction of peripheral and placental maternal tolerance of pregnancy.

G. Persson[1], C.S. Stæhr[1], F.S. Klok[1], J.B.S. Bork[1], C. Isgaard[1], M. Lebech[2], T.V.F. Hviid[1]

[1]Zealand University Hospital, Department of Clinical Biochemistry, Roskilde, Denmark ;
[2]Zealand University Hospital, Department of Gynaecology and Obstetrics, Roskilde, Denmark

Study question: How is the expression and the isoform profile of HLA-G regulated at the feto-maternal interface and systemically in the mother in uncomplicated pregnancy versus pre-eclampsia?
Summary answer: Levels of peripheral and placental soluble HLA-G (sHLA-G) are not correlated and may represent different compartments. HLA-G expression profile can be regulated directly by cytokines.
What is known already: The expression of paternally derived antigens by the fetal cells during establishment of pregnancy must involve induction of maternal tolerance. HLA-G expression by the endometrium and placental trophoblast cells is an important factor that allows generation of tolerance. HLA-G expression may be modulated by local factors such as hormones and cytokines (e.g. progesterone and IFN-γ). Implantation failure, recurrent abortion and pregnancy complications such as pre-eclampsia (PE) are often characterized by an altered HLA-G, cytokine and hormone profile. However, these measurements are often based on systemic serum levels. No clear picture of the local HLA-G regulation has yet been established.
Study design, size, duration: The direct effect of cytokines on HLA-G expression was studied *in vitro* by stimulating the chorioncarcinoma cell line JEG-3 with regulatory, anti- and pro-inflammatory cytokines. From October 2017 until present 30 women with uncomplicated pregnancies and 20 cases of PE diagnosed according to the Danish guidelines have been recruited at Zealand University Hospital, Denmark. Peripheral blood was collected pre-partum. Cord blood, placental biopsies and blood were collected within 30 minutes from birth.
Participants/materials, setting, methods: JEG-3 cells were stimulated for 24 or 72 hours with IL-2, IL-4, IL-6, IL-10, IL-12, IL-15, IL-17A, TGF-β_1, TNFα and IFN-γ1b. RNA was isolated from the placental biopsies, and serum and EDTA plasma were obtained from placental and maternal blood samples. Cord blood was used for HLA-G genotyping. HLA-G expression level was analyzed by flow cytometry, ELISA and digital droplet PCR. The mRNA isoform profile was analyzed using reverse transcription PCR and fragment analysis.
Main results and the role of chance: Peripheral and placental sHLA-G levels were not correlated. Cases with PE showed lower levels of peripheral sHLA-G, while no difference between groups was observed in placental sHLA-G levels. The expression pattern of the HLA-G mRNA isoforms were G1>G3=G2/4>G5=G6 for both the JEG-3 cell line and the placental biopsies. *In vitro* analysis showed that IL-2, IL-6 and TNF-α decreased expression of HLA-G, while IFN-γ upregulated its expression in JEG-3 cells. Interestingly, stimulation of JEG-3 with IL-10 changed the isoform profile, leading to an almost two-fold increase of the level of HLA-G1 over HLA-G2/4 and -G3 transcripts compared to the control. No differences in the isoform profile were observed between placental biopsies from uncomplicated pregnancies and PE placental biopsies, although cases of PE did have lower levels of HLA-G1. In ongoing analyses we are comparing the cytokine and hormone profile in the two patient groups and will correlate it to the level of HLA-G. Cell assays analyzing the effect of cytokine and hormone cocktails will be studied to evaluate the direct effect on HLA-G regulation.
Limitations, reasons for caution: As the JEG-3 cell line only represents an *in vitro* model for human trophoblast cells, it might not reflect *in vivo* settings. Also, cytokines co-exist within the local milieu and could have synergistic, antagonistic or indirect effects mediated through regulation of other immune factors on HLA-G expression *in vivo*.
Wider implications of the findings: This study provides a possible link between the increased levels of anti-inflammatory cytokines and reduced levels of sHLA-G. This could prove to be important for both early establishment of pregnancy and pregnancy complications such as PE. Also, systemic rather than placental inflammation might be involved in the manifestation of PE.
Trial registration number: not applicable

P-382 Evaluation of endometrial expression of cell adhesion genes in recurrent implantation failure (RIF) patients

A. Pathare[1], I. Hinduja[2]

[1]P. D. Hinduja Hospital and Medical Research centre, Department of IVF and Research, Mumbai, India ;
[2]P. D. Hinduja Hospital and Medical Research centre, Department of IVF, Mumbai, India

Study question: Does the endometrium of recurrent implantation failure (RIF) patients reveal differential expression of cell adhesion genes than fertile women in controlled ovarian stimulation (COS) cycle.
Summary answer: Out of seven cell adhesion genes evaluated in the study, five showed significant differential expression in endometrium of RIF patients than fertile women under COS.
What is known already: The cell adhesion molecules expressed by endometrium during window of implantation are proven to be important for endometrial receptivity. Based on our previously published whole genome microarray study, genes like CD300A, Cadherin-3 (CDH3), Cartilage Oligomeric Matrix Protein (COMP), Hyaluronan Binding Protein 2 (HABP2), Collagen Type XXII Alpha 1 Chain (COL22A1), Thrombospondin 1 (THBS1), Microfibril Associated Protein 4 (MFAP4) were associated with cell adhesion as one of the most significant biological functions among RIF patients during COS. Thus, it is worthwhile to study their expression in larger cohort of RIF patients under COS.
Study design, size, duration: Case-control study included RIF patients (n=25, cases) and healthy fertile oocyte donors (n=25, controls), both undergoing COS. The endometrial tissues were collected in the period of 2016-2019.
Participants/materials, setting, methods: Endometrial tissue was collected from cases and controls on Human chorionic gonadotropin (HCG)+7th day during COS. RNA extracted from tissue was converted to cDNA which was further subjected to real time PCR to study 'gene expression' by SYBR green chemistry. The differential expression of genes 'CD300A, CDH3, COMP, HABP2, COL22A1, THBS1, MFAP4' in cases were calculated by delta-delta-CT method, using fold change (FC) as parameter. Level of significance was determined using Mann-Whitney T test.
Main results and the role of chance: CDH3 (P=<0.0001, FC:-2.81), COMP (P=<0.0001, FC:-6.46), HABP2 (P=0.026, FC:-2.00), THBS1 (P=0.0238, FC:-1.70), MFP4 (P=0.0350, FC:-1.88) genes majorly involved in functions of cell adhesion like extracellular matrix organization, adhesion junctions in endometrium epithelium were observed to be significantly down-regulated with fold change > 2 in 'cases' compared to 'controls'
Limitations, reasons for caution: The study was performed in COS cycle, to rule out the hormonal bias. Therefore, the data cannot be generalized to overall cohort of RIF patients. Also, due to modest sample size of the study, it is necessary to be substantiated in larger population of RIF patients.
Wider implications of the findings: Down-regulation of cell adhesion panels in RIF patients indicate the impairment in endometrial receptivity which possibly contributes to repeated failure of implantation. On validating these markers in larger cohort of RIF patients, the panel can be implemented for assessment of endometrial receptivity to evaluate cell adhesion function in endometrium.
Trial registration number: Not applicable

P-383 Circulating miRNome during the implantation window as a non-invasive diagnostic tool to predict successful IVF/ICSI cycle

C. Baron[1], D. Haouzi[1], F. Entezami[2], S. Brouillet[3], S. Hamamah[3]

[1]Inserm U1203, IRMB- Hôpital St-Eloi- CHU Montpellier, Montpellier, France ; [2]American Hospital of Paris, IVF department, Neuilly-Sur-Seine, France ; [3]Arnaud de Villeneuve Hospital- CHU Montpellier, ART/PGD Department, Montpellier, France

Study question: Do circulating miRNAs profile during the implantation window under the same HRT treatment for frozen embryo replacement cycle, predict successful pregnancy ?

Summary answer: We identified a miRNA signature in serum during the implantation window that can predict successful pregnancy.

What is known already: The development of non-invasive tests predicting the pregnancy outcomes remains a challenge in assisted reproductive technology (ART). However, to date, very few studies analyzed the miRNome in bloodstream during the implantation window to identify potential circulating biomarkers related to the pregnancy outcomes.

Study design, size, duration: Serum (n=14) were collected during the implantation window from RIF patients (mean ± SD, age: 38.6 ± 5.0 years) under hormone replacement therapy (HRT) treatment for subsequent frozen embryo transfer. RNAs were extracted to perform the miRNA expression profile. Then, miRNA expression was analyzed according to the following pregnancy outcome: successful implantation (n=7) or implantation failure (n=7). Successful implantation was defined as both positive b-hCG and clinical pregnancy and implantation failure as negative b-hCG.

Participants/materials, setting, methods: Serum were obtained during a mock HRT treatment cycle from 14 repeated implantation failure (RIF) patients. Then, miRNA expression profile between groups, successful *vs*. implantation failure after frozen embryo replacement was evaluated by NGS using the HTG EdgeSeq miRNA Whole Transcriptome Assay (Illumina, Firalis). miRNA sequencing data were normalized using the method recommended by HTG molecular diagnostic. Then, statistical analysis and receiver operating characteristic (ROC) analysis were applied to miRNA sequencing data.

Main results and the role of chance: We identified 44 miRNAs differentially expressed between groups with a fold change > 2 and a P-value < 0.05. All miRNAs were overexpressed in serum from patients with successful implantation. *Supervised hierarchical clustering of these 44 miRNAs showed a good segregation of serum samples from patients with and without a successful implantation. related to these 44 miRNAs, we note four of them which are members of the let-7 family [*miR-1 (x2.4, p = 0.017, AUC = 0.88), miR-2 (x2.2, p = 0.004, AUC = 0.94), miR-3 (x2.1, p = 0.001, AUC = 0.90), miR-4 (x2.1, p = 0.001, AUC = 0.98)]. The microRNA target filter function from Ingenuity software predicted that 1375 mRNAs were targeted by the let-7 family that are involved in cell invasion, proliferation, growth and survival via integrin subunit beta 3 (*ITGB3*), vimentin (*VIM*), B-cell CLL/lymphoma 2 like 1 (*BCL2L1*) that play a central role in endometrial receptivity acquisition and implantation process. These results might have potential clinical applications to develop a non-invasive diagnostic tool to predict successful implantation, to avoid endometrium biopsy and consequently, to increase IVF/ICSI success.

Limitations, reasons for caution: Further investigations with a larger number of patients are in progress to validate theses results.

Wider implications of the findings: Circulating miRNAs profile of the implantation windows during a cycle preceding frozen embryo transfer cycle can predict the successful implantation. This information is crucial and can lead to the development a prognostic tool of the attempt, opening new perspectives in the patient care management.

Trial registration number: ID: NCT04192396

P-384 Four-fold increase in live birth rate: proof of the benefits of window of implantation testing in RIF patients by a prospective multicenter trial

F. Entezami[1,2], D. Haouzi[2,3], A. Ferrières-Hoa[2,3], C. Vincens[3], S. Bringer-Deutsch[3], E. Vintejoux[3], S. Hamamah[2,3]

[1]American Hospital of Paris, IVF, Neuilly sur Seine, France ; [2]Université Montpellier- CHU Montpellier- Hôpital Saint Eloi, Inserm U1203-, Montpellier, France ; [3]Arnaud de Villeneuve Hospital- CHU Montpellier, ART-PGD department, Montpellier, France

Study question: What outcome benefits are expected in RIF patients after customized embryo transfer based upon identification of endometrial receptivity window by transcriptomic approach.

Summary answer: Customized embryo transfer according to window of implantation testing improves pregnancy and birth outcomes for RIF patients.

What is known already: A receptive endometrium is mandatory for successful implantation of a competent embryo. For years, endometrial receptivity assessment was established by indirect methods and several non-invasive approaches have been reported with controversial results and poor predictive value. Transcriptomic and proteomic approaches have also been used to define molecular signatures and identify specific biomarkers of human endometrial receptivity. We previously identified a set of genes as biomarkers of endometrial receptivity and developed an innovative test based on quantification by RT-qPCR of these biomarkers in endometrium biopsies and tested its relevance in fertile patient.

Study design, size, duration: Endometrial biopsies were performed during the implantation window 7-9 days after the LH surgein natural cycle or 5-9 days after progesterone administration under HRTrespectively. According to transcriptomic testing result in the subsequent frozen embryo transfer cycle (FRET), blastocysts were transferred at the specific day where endometrium was identified as receptive and cleavage stageD2/D3 embryos were transferred72/48 hours before the specific cycle day where endometrium was identified as receptive.

Participants/materials, setting, methods: During 2015-2018 a total of 217 RIF patients (4.4±1.9 failed fresh/frozen transfers of 6.4±3.6 embryos) were enrolled. Genomic testing of endometrial biopsies was performed under natural/HRTcycles and mRNA expression of genes predictive of receptivity were established by RT-qPCR. Customized embryo transfer (n=157 patients) was performed in a subsequent FRET cycle based upon these results. Clinical pregnancy and live birth rates (LBR) were compared to control RIF patients with standard FRET in natural/HRT cycles (n=60 patients).

Main results and the role of chance: Customized embryo transfer using the genomic testing strategy yielded spectacular increase in outcome for 157 RIF patients (age 37.2±4.3 years). Comparison of the results between the study group and the control group showed significantly higher rates for implantation (22.7% vs 7.2%, p=0.0001), clinical pregnancy (38.8% vs 15.0%, p=0.0006), ongoing pregnancy (36.3% vs 8.3%, p=0.00002) and live birth rates (31.8% vs 8.3%, p=0.0002). Analyses of endometrial receptivity status revealed mostly a delay both in natural and HRT cycles between 1 to 3 days. Most patients achieved pregnancy after the first customized FRET (70%). Better clinical pregnancy and live birth rates were obtained with blastocyst transfer compared to cleavage stage transfers (respectively 36.3% vs 22.5% and 29.5% vs 14.0%). Whatever the day of transfer, results were significantly higher in the study group compared to the control group, with ongoing pregnancy rate for transfer of cleavage-stage embryos of 21.1% compared to 4.5% in the control group (p=0.03). In blastocyst transfer group ongoing pregnancy rate of 33.9% was significantly greater than 8.6% in the control group (p=0.003).

Limitations, reasons for caution: The benefit of this innovative strategy should be analysed with respect to the genetic status of the transferred embryos after PGT for aneuploidy.

Wider implications of the findings: In RIF patients customized embryo transfer according to endometrial transcriptomic testing improves dramatically the clinical outcome by increasing almost four-fold the LBR. In our study, the majority of RIF patients displayed a delay in their receptivity window of 1- 3 days, revealing a potential cause for their previous implantation failures.

Trial registration number: NCT04192396

P-385 Hysteroscopic endometrial peeling: a different approach to endometrial scratching

T. Alkon[1], M. Luna-Rojas[1], C. Hernandez-Nieto[1], D. Cassis-Bendeck[1], B. Sandler[1]

[1]Reproductive Medicine Associates of New York, Reproductive endocrinology and Infertility, Mexico City, Mexico

Study question: Does hysteroscopic endometrial peeling improve reproductive outcomes in women with implantation failure?
Summary answer: Women with implantation failure may benefit from intraoperative endometrial peeling prior to a frozen embryo transfer (FET).
What is known already: Trials on endometrial scratching with pipelle and diagnostic hysteroscopies in women with implantation failure have demonstrated methodological limitations and high risk of bias, making it unclear whether these procedures improve reproductive outcomes. To date, no study has described the effects of intentional endometrial lesion through hysteroscopy.
Study design, size, duration: A retrospective, cohort study included infertile patients who failed transfers of at least two good quality blastocysts. Thereafter, underwent an endometrial peeling through hysteroscopy and subsequently a blastocyst FET from January 2018 to December 2019. A sample size of 70 patients per group was calculated to be necessary to detect a 15% difference in clinical pregnancy rates with 80% power. Alpha=0.05.
Participants/materials, setting, methods: Women <40 years, with implantation failure, normal saline sonogram and, no previous uterine surgeries were segregated into two groups: A) 70 patients underwent hysteroscopic endometrial peeling, which consists on removing the superficial endometrial layer of the whole uterine cavity with hysteroscopic biopsy forceps and B) 85 controls who did not undergo surgical endometrial peeling. All patients underwent a subsequent blastocyst FET. Trophectoderm biopsy cases were excluded from the analysis.
Main results and the role of chance: In total, 155 women were included in the cohort. No differences were found in age, body mass index, baseline FSH, AMH, baseline antral follicle count, previous number of stimulation/IVF cycles, number of embryos transferred and, embryonic quality among cohorts. Evaluating the endometrium, no differences were observed in the endometrial pattern, however, a significant difference in the endometrial thickness (8.5±1.4mm vs 9.0±1.6mm, p=0.03) was noted among groups. When analyzing the subsequent FET cycle, statistical significance was observed in implantation rates (38.8% vs 16.6%, p= 0.01) and clinical pregnancy rates (65.7% vs 30.3%, p=0.0009). No difference was found in clinical loss rates (10.4% vs 17.1%, p=0.08) among Group A vs Group B respectively.

Of the patients who underwent surgical endometrial peeling, 15.7% (11/70) had the following incidental intraoperative findings: mild intrauterine and/or cervical adhesions (4/70, 0.05%), endometrial polyps (8/70, 11.4%), polypoid endometrium (6/70, 0.8%). 63.6% of the patients with incidental findings became pregnant.
Limitations, reasons for caution: This study is limited by its retrospective nature. Additionally, in patients with noted uterine pathology, appropriate surgical management was administered in the same setting, this could have biased the results. Future randomized controlled trials are needed with euploid frozen embryo transfers.
Wider implications of the findings: This study describes for the first time the clinical utility of mechanically peeling the superficial endometrial layer through hysteroscopy in women with implantation failure. Our findings suggest that surgically removing superficial endometrial tissue, promotes the generation of new receptive tissue, favoring embryo implantation.
Trial registration number: Not applicable

P-386 HCG improves pregnancy chances in embryo transfers using euploid embryos and Gestational Carriers after one implantation failure

J. Mashiach[1], A. Roumia[1], M. Dviri[2], N. Weizman[1], S. Baram[1], C. Librach[3]

[1]CreATe Fertility Center, CreATe Fertility Center, toronto, Canada ;
[2]CreATe Fertility Center, CrATe Fertility center, Toronto, Canada ;
[3]CreATe Fertiity Center- Unviversity of Toronto, Obstetrics and Gynecology, Toronto, Canada

Study question: Does intrauterine hCG improve pregnancy rate in frozen single embryo transfer of euploid embryos in Gestational Carriers (GC)?
Summary answer: After a first failed euploid single embryo transfer into a GC, intrauterine administration of hCG appears to improve the chances of subsequent implantation and pregnancy.
What is known already: Implantation is considered the "black box" in ART. Implantation is a highly regulated process that requires: receptive endometrium, functional blastocyst, and interaction between them. Human chorionic gonadotropin (hCG) is a hormone synthesized and released by the syncytiotrophoblast. It stimulates the ovarian production of progesterone during the first trimester of pregnancy. Several studies suggest that intrauterine hCG increases the pregnancy rate. The hCG may promote peritrophoblastic immune tolerance, which facilitates trophoblast invasion by inducing an increase in endometrial T-cell apoptosis. It may also support trophoblast apposition and adhesion by regulating proteins involved in implantation as well as altering endometrial secretory parameters.
Study design, size, duration: We retrospectively reviewed all single euploid frozen embryo transfers (FET) of euploid embryos into GCs at the CReATe Fertility Center between Jan 2017 and Mar 2019. Mosaic embryos were excluded. We compared the cycles in which 50 µL of hCG was introduced into the uterine cavity 10-15 min prior to embryo transfer vs. cycles where no hCG was administered. We then compared the first transfer and all transfers after a failed transfer in both groups.
Participants/materials, setting, methods: We analyzed FET cycles of Euploid embryos in GCs. The GC's underwent a pelvic US and sonohysterogram before ET. The patients received HRT for uterine preparation. hCG insertion was used in the transfer cycle at the discretion of the fertility physician.
Main results and the role of chance: First transfer

	With hCG n(%)	Without hCG n(%)	P Value
Age	26.44±4.78	27.62±4.47	.289
Endometrial thickness	10.8±2.02	10.43±2.02	.52
Morphology			.350
1	6/18 (33.3%)	29/196 (14.8%)	
2	9/18 (50%)	128/196 (65.3%)	
3	2/18 (11.1%)	28/196 (14.3%)	
Egg donation	15/18 (83.3%)	152/196 (77.6%)	.57
Adjunct therapy	18/18 (100%)	153/196 (78.1%)	<.001
Pregnancy	13/18 (72%)	132/195 (67%)	.69
Clinical Pregnancy	13/18 (72%)	119/195(61%)	.87

After at least 1 failed transfer

	With hCG n(%)	Without hCG n(%)	P Value
Age	26±3.67	27.9±4.61	.035
Endometrial thickness	10.81±2.27	9.92±2.27	.52
Morphology			.51
1	4/34 (11.7%)	16/111 (14.4%)	
2	23/34 (67.6%)	57/111 (51.3%)	
3	4/34 (11.7%)	25/111 (22.5%)	
Egg donation	30/34 (88.2%)	82/111 (73.8%)	.21
Adjunct therapy	31/34 (91.1%)	60/111 (54%)	<.0001
Pregnancy	21/34(61.7%)	59/111 (53%)	.02
Clinical pregnancy	20/34(58.8%)	44/111(40%)	.04

Using a logistic regression model, accounting for age at OPU, adjunct therapy and intrauterine hCG administration, in those with 1 or more previous failed

transfers, we observed that intrauterine hCG administration was significantly associated with the chance of achieving pregnancy (CI 1.048-6.57 p=0.039).
Limitations, reasons for caution: The retrospective nature of the study and the relatively small study size are the main limitations of this study.
Wider implications of the findings: In our model of euploid embryos in "proven" uteri of GCs, we can investigate implantation in the most optimal clinical setting possible. When implantation of a euploid embryo fails (i.e failed first transfer), intrauterine administration of hCG appears to increase the chance for pregnancy.
Trial registration number: n/a

P-387 Endometrial receptivity - Is it possible to make the "old" research methods more informative?

I. Gyulmamedova[1], T. Zadorozhna[2], T. Tatarchuk[3], O. Girina[4], N. Vladykina[4], M. Sokolova[5], O. Gyulmamedova[6], V. Sosnina[7], O. Nosenko[8]

[1]Adonis- Medical Group of Companies- Kyiv- Ukraine, Iivf, Kyiv, Ukraine ;
[2]Institute of Pediatrics Obstetrics and Gynecology- National Academy of Ukraine, Laboratory of Morphology, Kyiv-, Ukraine ;
[3]Institute of Pediatrics Obstetrics and Gynecology- National Academy of Ukraine, Department of Endocrine Gynecology-, Kyiv-, Ukraine ;
[4]Adonis- Medical Group of Companies- Kyiv- Ukraine, ivf, Kyiv, Ukraine ;
[5]Adonis- Medical Group of Companies-, ivf, Kyiv-, Ukraine ;
[6]Medical Center IPF-, ivf, Kyiv-, Ukraine ;
[7]Medical Center IPF, -ivf-, Kyiv-, Ukraine ;
[8]Odessa National Medical University, Department of Obstetrics and Gynecology, Odessa, Ukraine

Study question: Is the proposed examining technology of endometrium receptivity informative in women with Recurrent Implantation Failure (RIF)?
Summary answer: The complex application of the morphological, immunohistochemical methods, scanning electron microscopy is informative and effective for the diagnosis of endometrial receptivity in women with RIF.
What is known already: Single competent blastocyst transfer in maximum endometrium receptivity is the key goal for reproductive medicine. Knowledge of the implantation window (IW, is especially important in patients with recurrent implantation failure (RIF). Significant efforts are focused on the search and implementation of non-invasive endometrial receptivity biomarkers - proteomics and lipidomics in intrauterine fluid in the cycle of embryo transfer (ET). Gene-array methods are used to analyze the global gene profile of endometrial cells. However, in the daily work, morphological research and endometrium dating, as well as scanning electron microscopy (SEM), continue to be of clinical interest due to their availability.
Study design, size, duration: During 2010-2019 In 77 patients with RIF on the 7-10th day of the menstrual cycle, an endometrial biopsy / hysteroscopy was performed. A morphological , immunohistochemical (CD-138) study was performed; After correction of the revealed disorders, in the following cycles, the endometrial biopsy was obtained at P+6; P+8 or P + 7; P + 9 day of exposure to progesterone. A morphological study (MS) and SEM were carried out. Descriptive statistics methods used.
Participants/materials, setting, methods: The age of the patients - 34.8± 0.59,, the duration of infertility 8.48 ± 0.64 , the number of IVF attempts 3,11 ± 0.19. According to the results of MS, patients were divided: **Gr1** - 13 (16,88%) - the endometrium without morphological abnormalities.**Gr2** - Chronic endometritis with micro- macropolyposis, confirmed by CD 138 – 19 (24, 68 %); **Gr3** - Stromal fibrosis, endometrial hypoplasia - 29 (37,66%).**Gr4**- dyschronosis of glands and stroma - 16 (20,78%)
Main results and the role of chance: Not informative results (NIR), repeated biopsy (RB) at a later date after additional treatment : Gr 2 - 6 (31,58 %) , Gr3 - 3 (10,34%); Gr 4 - 2 (11.1%)**The reason NIR**: Dense film, an abundance of red blood cells, absence pinopodia - P + 6; P + 8; Gr1 - no indication for a RB (P1-2<0.02). In all 11 patients with RB, temporary trends in the formation of pinopodia persisted **Few pinopodia:** Cr1 - 1 (7,69%) , Gr 2 - 12 (63,16%); Gr 3- 16 (59%), Gr 4 – 6 (37,5%) patients (P <0.04;1-2,1-3,1-4) **Dyschronosis of the development of pinopodia**: 1 (7,69 %), 2 (10,53%), 3 (11,11%), 14 (87,5 %) respectively (P 1-4 <0.001) **The lag of the formation of pinopodia** for 2 or more days: 8 (61,54%) , 13 (68,42%) , 19 (65,52%), 5 (31,25%) respectively (P> 0.05; 1-2,1-3.1-4). ***Lack of regression, polymorphism of microvilli;*** *Gr 1 -0; Gr 2* - 2 (10,53%) ; Gr 3 - 7 (25,00%) , Gr 4 – 9 (56,25%) *(* P 1-3 <0.03; P 1-4 <0.001).After correction of the revealed disorders in women with RIF– ET according to the implantation window was performed pregnancy rate - 48 (62,34 %)
Limitations, reasons for caution: Gr1 the main reason of RIF is the peculiarity of IW, expected to have a high clinical effect using genetic methods. Gr 2, Gr3 clinical efficacy depends not only on IW, but also deviations in the structure of the endometrium. Gr1 -dyschronosis is the most complex and needs further research.
Wider implications of the findings: The proposed examining technology of endometrium receptivity helps to obtain pregnancy in complex category of patients with RIF. The proposed examining technology remains an informative, reproducible, accessible method. The technology help determine the indications for molecular genetic studies of the endometrium and interpret the results
Trial registration number: not applicable

P-388 Significance of invasion of decidualized endometrial stromal cells by human embryonic stem cells derived trophoblastic spheroids (BAP-EB) in pregnancy outcomes of in vitro fertilization (IVF)

Y.L. Lee[1,2], S.W. Fong[1], K.C. Lee[1], A.C.H. Chen[1,2], K.F. Lee[1,2], W.S.B. Yeung[1,2], E.H.Y. Ng[1,2]

[1]The University of Hong Kong, Obstetrics and Gynaecology, Hong Kong, Hong Kong ;
[2]The University of Hong Kong Shenzhen Hospital, Shenzhen Key Laboratory of Fertility Regulation, Shenzhen, China

Study question: Does invasion of decidualized endometrial stromal cells (hEnSC) towards trophoblast-like BAP-EB relate to IVF pregnancy outcome?
Summary answer: The invasion of decidualized hEnSC from patients with a live birth towards BAP-EB was significantly higher than those with negative pregnancy outcome.
What is known already: The success rate of IVF remains low even after the transfer of good quality embryos. Implantation-competent blastocysts can induce migration of decidualizing hEnSC. The encapsulation of the invading blastocyst by the decidua supports subsequent trophoblast invasion. The study of the dynamic fetal–maternal interaction between the decidua and the implanting blastocyst requires a proper implantation model. We have derived trophoblastic spheroids (BAP-EB) from human embryonic stem cells that resemble human trophectoderm and trophoblast during early implantation process. They attach specifically onto receptive endometrial epithelial cells. BAP-EB can be used as human embryo surrogate for studying the early implantation process.
Study design, size, duration: hEnSC were isolated from endometrial biopsies obtained from IVF patients in their natural cycle 7 days after luteinizing hormone surge (LH+7). Human embryonic stem cells were differentiated into early trophectoderm-like BAP-EB-48h or trophoblast-like BAP-EB-96h. An invasion assay using Matrigel Invasion Chamber was established with decidualized hEnSC and BAP-EB-48h or -96h. hEnSC isolated from patients with live births (n=5) and negative pregnancy outcomes (n=5) in their subsequent stimulated IVF cycle were compared.
Participants/materials, setting, methods: Human endometrial stromal cell line (T-HESC) was induced to decidualize in vitro. Purity of primary hEnSC was confirmed by immunohistochemical staining of stromal (Vimentin) and epithelial (cytokeratin) markers. The 24h-invasion of decidualized hEnSC through Matrigel towards trophectoderm-like BAP-EB-48h or trophoblast-like BAP-EB-96h was compared to medium alone control. The expression levels of decidualization markers (PRL, IGFBP1) and the invasion ability towards BAP-EB were compared between hEnSC isolated from patients with live births and negative pregnancy outcomes.
Main results and the role of chance: After treatment with cAMP for 3, 6 and 9 days, the decidualization markers PRL and IGFBP1 were significantly induced from day 6 onwards. The isolated primary hEnSC were of high purity as demonstrated by positive vimentin staining (>95%) but not cytokeratin staining. The invasion of hEnSC through Matrigel towards medium alone without BAP-EB, BAP-EB-48h or BAP-EB-96h was quantified by the absorbance of crystal violet stained cells. It was found that significantly more hEnSC invaded towards BAP-EB-96h than BAP-EB-48h when compared to medium alone control, suggesting the specific invasion of hEnSC towards the trophoblast-like but not the trophectoderm-like BAP-EB. The decidualization and invasion abilities of hEnSC

isolated from patients with live births and negative pregnancy outcomes were compared. The expression levels of PRL and IGFBP1 were similar in the two groups of patients 6 days after in vitro induction of decidualization (p>0.05). The invasion of hEnSC through Matrigel in the presence or absence of BAP-EB-96h was measured. Interestingly, the percentage of BAP-EB-96h-induced invasion was significantly higher in hEnSC from patients with live births (54.3% ±11.1%) than those with negative pregnancy outcomes (20.8%±9.1%, p<0.05). The data indicated a correlation of responsiveness of hEnSC towards BAP-EB with pregnancy outcome.

Limitations, reasons for caution: BAP-EB and the isolated endometrial stromal cells may not fully represent the in vivo developed human blastocysts and endometrial cells, respectively. The in vitro nature of the hEnSC experiments and the limited sample size in this study may also limit the interpretation of the data.

Wider implications of the findings: The study of early implantation failure is limited by the number and the ethical concerns of human embryos donated for research. The current data demonstrated the potential use of BAP-EB as human embryo surrogate for assessing the responsiveness of hEnSC towards implanting embryos and as predictive tool for pregnancy outcome.

Trial registration number: nil

P-389 Maternal germline factors associated with aneuploid pregnancy loss: a systematic review.

U. Blyth[1], L. Craciunas[2], M. Choudhary[3]

[1]Newcastle upon Tyne Hospitals NHS Foundation Trust, Academic Foundation Programme, Newcastle upon Tyne, United Kingdom ;

[2]Newcastle upon Tyne Hospitals NHS Foundation Trust, Obstetrics and Gynaecology, Newcastle upon Tyne, United Kingdom ;

[3]Fertility Centre at Life- Newcastle upon Tyne Hospitals NHS Trust- Biomedicine West Wing- Newcastle upon Tyne- NE1 4EP- UK, Reproductive Medicine, Newcastule upon Tyne, United Kingdom

Study question: Which maternal germline factors have been associated with pregnancy loss secondary to numerical chromosome abnormalities of the embryo?

Summary answer: A range of maternal germline factors such as insertions, deletions, translocations, copy number variants or single gene mutations have been associated with aneuploid pregnancy loss.

What is known already: Miscarriage describes the spontaneous loss of pregnancy before the threshold of viability; the vast majority occur before 12 weeks of gestation. Miscarriage affects 1 in 4 couples and is the most common complication of pregnancy. Chromosomal abnormalities of the embryo are identified in approximately 50% of first trimester miscarriages; aneuploidy accounts for 86% of these cases.

The majority of trisomic miscarriages are of maternal origin with errors during meiotic division of the oocytes. Chromosome segregation errors in oocytes may be sporadic events secondary to advancing maternal age; however, there is increasing evidence to suggest possible maternal germline contributions to this.

Study design, size, duration: A systematic review of the literature was conducted to identify maternal germline factors associated with pregnancy loss secondary to numerical chromosome abnormalities of the embryo. The literature search was run in September 2019 using the electronic databases: OVID MEDLINE, EMBASE and the Cochrane Library. No time or language restrictions were applied to the searches, only primary research was included. The review protocol was registered with PROSPERO (CRD42019153653) following PRISMA guidelines.

Participants/materials, setting, methods: Participants were women who had suffered pregnancy loss secondary to numerical abnormalities of the embryo. Study identification and subsequent data extraction was performed by two authors independently and in duplicate. The Newcastle-Ottawa Scale was used to judge included studies on three broad perspectives: the selection of study groups; the comparability of the groups; and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies respectively. The results were synthesised narratively.

Main results and the role of chance: The literature search identified 2639 titles, out of which 54 were eligible for inclusion in this systematic review. They reported on maternal germline factors having variable degrees of association with pregnancy loss of aneuploid origin. The AmiGO gene ontology database was used as a reference to establish the functional role currently attributed to the genes reported.

Structural factors inherited from mothers directly by embryos were the majority of the cases reported and included: Robertsonian translocations, deletions and insertions. Cases of maternal mosaicism leading to aneuploidy in the embryo included: trisomy 9 and duplication of chromosome 4p. Several of these cases were identified using FISH in addition to traditional karyotyping techniques.

Germline factors with a plausible role in aneuploid pregnancy loss of maternal origin included: skewed X-inactivation and FMR1 repeats.

Studies that reported the association of single gene mutations with aneuploid pregnancy loss were conflicting. Single gene mutations with an uncertain role in aneuploid pregnancy loss included: SYCP3 and MTHFR. Both genes have an established role in cell division in animal models however the research in humans is inconclusive.

One study reported an association of a single gene mutation of NLRP7 in women with recurrent molar pregnancies.

Limitations, reasons for caution: Whilst this review was based on a broad systematic search of the literature most studies identified were case reports: only a fraction were cohort studies of moderate quality. The role of paternal germline factors in aneuploid pregnancy loss was not addressed in this systematic review and warrants future research.

Wider implications of the findings: Identifying maternal genetic factors associated with an increased risk of aneuploidy will expand our understanding of cell division, nondisjunction and miscarriage secondary to embryo aneuploidy.

The candidate germline factors identified may be incorporated in a screening panel for women suffering miscarriage of aneuploidy aetiology to facilitate counselling for subsequent pregnancies.

Trial registration number: PROSPERO registration number CRD42019153653

P-390 Pattern of early beta human chorionic gonadotropin (β-hCG) in intracytoplasmic sperm injection (ICSI) for pregestational testing for monogenic disorders (ICSI/ PGT-M) versus ICSI only cycles

N. Samara[1], E. Haikin-Herzberger[1], A. Dick[1], H. Amir[1], A. Reches[1], M. Malcov[1], D. Ben Yosef[1], F. Azem[1]

[1]lis maternirty hospital- Sourasky medical center.affiliated with the Sackler Fac, Wolf PGD Stem Cell Lab- Racine IVF Unit, Tel Aviv, Israel

Study question: Is there a difference in β-hCG pattern in pregnancies achieved following ICSI/PGT-M versus ICSI?

Summary answer: A comparable pattern of early β-hCG rise were observed in ICSI/PGT-M cycles compared with ICSI only.

What is known already: Initial serum β-hCG level is used to confirm pregnancy following IVF, scheduled follow-up testing is accepted to reassure normal progression and prediction of outcome. β-hCG levels and pattern of increase are well correlated with pregnancy outcome. In our unit, fertilization in PGT-M cycles is routinely achieved by ICSI, to avoid sperm contamination of analysis result. On day 3 or 5, embryonic biopsy is performed for genetic analysis, which might affect syncytiotrophoblast secretion of β-hCG. Our aim was to assess early β-hCG rise in ICSI/PGT-M compared to ICSI cycles in order to evaluate possible effect of biopsy on early β-hCG rise

Study design, size, duration: This retrospective cohort study was performed at a tertiary university-affiliated medical center. It included patients with single embryo transfer, from ICS/PGT-M and ICSI only cycles, performed in our IVF unit between June 2017 and September 2018. Both fresh and frozen embryo transfer (FET) cycles were included. A total of 163 cycles with a positive β-hCG result were available for analysis, of which 85 of ICSI/PGT-M and 78 of ICSI

Participants/materials, setting, methods: In ICSI/PGT-M cycles embryo biopsy took place on day 3 or 5 according to genetic analysis method, control group included patients undergoing ICSI during same period. First β-hCG was timed on day 10-12 according to transfer day and second test was usually taken two days later. In order to adjust for different period of time between the two tests and for the none-linear elevation of Beta-HCG we used a calculated parameter - ln (Delta-Beta-HCG/period).

Main results and the role of chance: The two groups were comparable with respect to age, BMI and parity. Gravidity was significantly higher in the ICSI/PGT-M than in ICSI group (2.0±1.8 vs.0.8±1.1, p<0.01). In the majority of PGT-M cycles embryos were transferred on day 5 and none were transferred on day 3, compared to ICSI cycles in which the majority of embryos were transferred on day 3, in ICSI/PGT-M cycles some embryo were transferred on day 4 shortly after receiving genetic analysis results (61.9% vs 33.8% for day 5-6, 38.1% vs 1.3% for day 4 and 0% vs. 64/9% for day 2-3, p<0.01). Once β-hCG was positive, the clinical pregnancy rates were 98.2% in the ICSI/PGT-M group compared to 92.2% in the ICSI group. Similarly, live birth rates (65.8% in the PGT-M vs. 72%% in ICSI) were not significantly different. Differences between serum β-hCG levels were not statistically significant between two groups in both first and second tests, neither when comparing the ln(delta-β-hCG/period). After adjustments for age, number of previous pregnancies, fresh vs. frozen and embryo day of transfer, ln(delta-Beta-hCG/period) was still comparable between ICSI/ PGT-M group and ICSI group.

Limitations, reasons for caution: Our study is a retrospective one, with potential biases inherent to its design. In some cases, lag time between first and second β-hCG levels was not constant due to scheduling constraints.

Wider implications of the findings: Our study shows no difference in the early pattern of serum β-hCG levels between ICSI/PGT-M cycles and ICSI cycles. It reassures that embryo biopsy for ICSI/PGT-M, taken at day 3 or 5 in both fresh and frozen cycles, does not affect early β-hCG rise, correlated with pregnancy outcome.

Trial registration number: not applicable

P-391 Pregnancy viability in relation to demographic and repeated sonographic and serum biomarkers

J.F. Petersen[1], L. Friis-Hansen[2], T. Bryndorf[3], A.K. Jensen[4], A. Nyboe Andersen[5], E. Løkkegaard[1]

[1]North Zealand Hospital, Obstetrics and gynecology, HIllerød, Denmark ; [2]North Zealand Hospital, Clinical Biochemistry, Hillerød, Denmark ; [3]Private Practice, Gynecological clinic, Copenhagen, Denmark ; [4]Institute of Public Health- University of Copenhagen, Section of Biostatistics, Copenhagen, Denmark ; [5]Rigshospitalet, The Fertility Clinic, Copenhagen, Denmark

Study question: Which individual and combined variables of demography, transvaginal sonography and serum markers are the best dynamic predictors of viability in the first trimester?

Summary answer: Combined maternal age, hCG, crown-rumP-length and bleeding data was the best model to dynamically predict miscarriage, closely followed by maternal age, hCG, estradiol and bleeding

What is known already: Early pregnancy losses are common and about 25% of recognized pregnancies end in a miscarriage. More precise prediction of miscarriage could potentially alleviate the anxiety of couples experiencing symptoms but remains challenging. The diagnosis often depends on the development of ultrasonic or biochemical evidence. Maternal age is the principal risk factor and the current tendency towards delayed childbearing leads to an increased incidence of clinical visits for viability reassurance. Early pregnancy diagnostics is usually based on retrospective demographic, ultrasonic or biochemical data. Potentially, all three sources prospectively combined carries an increased accuracy compared with any one-parameter diagnostics

Study design, size, duration: A prospective cohort of pregnant women with asymptomatic and assumed healthy pregnancies of no more than 55 days' gestation was recruited and followed from June 2016 to March 2017 at two University Hospitals in Copenhagen, Denmark. Participants had repeated visits every second week from 4–14 weeks' gestation until either miscarriage or confirmed viability at the end of the first trimester. The study population included 203 women with 715 visits

Participants/materials, setting, methods: Before inclusion, participants and male partners completed a validated questionnaire for baseline demographic data. At each visit, a panel of 29 commonly used blood-samples, transvaginal sonography and symptoms of miscarriage was recorded. Based on updated values, unadjusted hazard ratios (HR) identified the eight most influential biomarkers on the risk of miscarriage. Testing these in adjusted groups of three, 56 different combinations were evaluated for their ability to individually and dynamically predict viability at each visit

Main results and the role of chance: From adjusted odds ratios (aOR) of demographic factors, older (aOR 1.2, 95%CI [1.1; 1.3], p < 0.01) or obese (>35kg/m^2) women (aOR 3.4, 95%CI [1.1; 10], p = 0.03) had increased risks of miscarriage. Risk was reduced after having previously delivered >2 babies (aOR 0.1, 95% CI [0.01; 0.6], p = 0.02). Regarding the serially collected data, maternal age remained a risk factor for miscarriage especially in women aged ≥35 years (HR 2.6, 95%CI [1.2–5.6]). Ultrasonical alterations in crown-rumP-length, mean gestational sac diameter or presence of bleeding were also important (HR 0.76, 95%CI [0.69–0.82], HR 0.72, 95%CI [0.64–0.79] and HR 1.13, 95%CI [1.03–1.27], respectively). Biochemically, serum hCG, progesterone and estradiol, respectively showed the most distinct effect with hazard ratios of 0.36, 95%CI [0.26–0.48], 0.76, 95%CI [0.75–0.77]and 0.80, 95%CI [0.75–0.84], respectively. From the tested 56 different combinations, a model including maternal age (>35 years or not), bleeding (yes/no), CRL (mm) and hCG (U/L) data was the best to predict miscarriage (WAIC score 3118, differences were evaluated by the statistical Watanabe-Akaike information criterion (WAIC)). The best combination without sonographic data (WAIC 3424) was the combination of maternal age, bleeding, hCG and estradiol

Limitations, reasons for caution: Fetal karyotype was unavailable due to logistics and heart rate frequency was not measured due to risk of doppler heat-induced damage. Because of the demands of serial data collection, the sample size was too small for other estimates than the WAIC for the quality of prediction in each model

Wider implications of the findings: Reassuring, the widely applied approach of combining hCG, CRL and bleeding data for early pregnancy outcome turned out to be the best. However, without sonography the best model had nearly the same ability. Future trials might explore this combination in women with pregnancies <7 weeks' gestation or unknown location

Trial registration number: NCT02761772

P-392 Extracellular vesicles: an important biomarker in recurrent pregnancy loss?

N. Rajaratnam[1], N.E. Ditlevsen[2], M.M. Jørgensen[3], O.B. Christiansen[4]

[1]Aalborg University Hospital, Emergency- and Trauma Center, Aalborg, Denmark ; [2]Region Hospital North, Emergency Department, Hjoerring, Denmark ; [3]Aalborg University Hospital- The Faculty of Medicine, Clinical Immunology and Clinical Diagnostics, Aalborg, Denmark ; [4]Aalborg University Hospital- The Faculty of Medicine, Obstetrics and Gynaecology, Aalborg, Denmark

Study question: Can different types of extracellular vesicles (EVs) markers be used as predictive markers of pregnancy outcome in recurrent pregnancy loss (RPL) patients?

Summary answer: Although tendencies were found, there were no significant differences in the progression of EV levels throughout pregnancy between RPL patients with live birth or miscarriage.

What is known already: RPL defined as ≥ 3 miscarriages has an estimated incidence of 1-3% in all couples. The etiology of RPL is considered to be multifactorial. EVs are small particles secreted into plasma that take part in numerous physiological processes and their contents provide information about the originating cell and pathophysiological states in different diseases. In pregnancy disorders, changes can be seen in the composition, bioactivity and concentration of placental and non-placental EVs. So far no laboratory test has a good diagnostic value in predicting new pregnancy loss in RPL patients.

Study design, size, duration: Prospective cohort study performed at a tertiary university-affiliated centre for RPL from the period July 2018-June 2019. The study includes 26 pregnant RPL patients, n=20 with live birth (LB) and n=6 with a new miscarriage (MI). Within these groups 4 patients in the MI group received intravenous immunoglobulin (IVIG) as treatment, and 8 in the LB group. The decision to provide IVIG was mainly made on the basis of a high number of previous miscarriages.

Participants/materials, setting, methods: Up to 5 blood samples were obtained from each patient: 1 baseline before pregnancy and 1-4 during pregnancy in gestational week 6, 8, 10 and 16. The samples were analyzed for EV type and concentration with EV Array, a method able to capture EVs by using an antibody panel targeting membrane proteins. 32 different markers were

analyzed specific for immunology/inflammation, coagulation, hypoxia, placenta and hormones.
Main results and the role of chance: The analysis showed that within the LB group (n=20, IVIG n=8, no IVIG n=12) there was a statistically significant (from $p < 0.01$ to $p < 0.0001$) increase in 47 % of the EV subtypes in the group receiving IVIG, between the sample taken before pregnancy and IVIG treatment and the sample taken at gestational week 6 after initiation of IVIG. Regarding pregnancy outcome, several graphs were made with median values for the LB group compared with graphs for each of the 6 miscarried pregnancies, These showed clear tendencies towards different progressions of the EVs positive for markers CD9, CD81, Annexin V (general EV markers), CAXII (hypoxia marker), FSHR, LHR (hormone receptors), TRAIL (immunological marker) and PLAP (placental marker). One patient with miscarriage of a normal but severely growth-restricted fetus in gestational week 21 already exhibited a marked decline of almost all EV subtypes from week 12.
Limitations, reasons for caution: A limitation is the small study size. It is unclear which changes in EV levels are induced by the growing, and in some cases probably ischemic placenta and which are exclusively due to the IVIG administration.
Wider implications of the findings: This is the first study of EVs in pregnancies of RPL patients.In a larger study it will be possible to investigate whether the clear tendencies for different progressions of EV subtypes during pregnancies with successful and unsuccessful outcomes still applies and whether it can be used as a prognostic biomarker.
Trial registration number: Ethics committee approval number: N-20180025

P-393 Relationship between the percentage of endometrial senescent cells and uterine natural killers (uNK) cells during the mid-luteal phase in women with recurrent pregnancy failure

D. Parvanov[1], R. Ganeva[1], N. Vidolova[1], G. Stamenov[2]

[1]Nadezhda Women's Health Hospital, Research, Sofia, Bulgaria ;
[2]Nadezhda Women's Health Hospital, Obstetrics and Gynecology, Sofia, Bulgaria

Study question: Is there an association between the presence of $p16^{INK4a}$-positive cells in different endometrial compartments and CD56+ uNK cells during the mid-luteal phase in RIF patients?
Summary answer: The percentage of endometrial stromal $p16^{INK4a}$-positive cells positively correlates with the abundance of CD56+ uNK cells in RIF women during the mid-luteal phase.
What is known already: $P16^{INK4a}$ is commonly measured in order to assess the level of senescence in the human endometrial tissue. Recent studies have shown that p16-positive senescent cells are involved in endometrial receptivity and in the acute cellular remodelling during the embryo implantation period. Recognition and elimination of these senescent cells by immune cells, especially CD56+ uNK cells, play an essential role in tissue repair and homeostasis. However, data about the association between the abundance of senescent cells and uNK cells in human endometrium is still scarce.
Study design, size, duration: This is an observational study of 92 women with recurrent implantation failure (RIF) who had an endometrial biopsy during the mid-lutheal phase. We used immunohistochemical (IHC) markers, p16 and CD56, in order to analyze the association between senescent cells and uNK in human endometrium.
Participants/materials, setting, methods: Our study was performed at Nadezhda Women's Health Hospital, Bulgaria. Patient biopsies with known pregnancy outcome were retrieved from our tissue bank. Tissue sections were stained immunohistochemically for CD56 (a uNK-specific cell surface antigen) using CD56 antibody (E-AB-62136, Elabscience, USA), or p16 senescent biomarker using $p16^{ink4a}$ antibody (Master Diagnostica, Granada, Spain). The percentage of positively stained cells was calculated after enumeration by two independent investigators in multiple endometrial sections.
Main results and the role of chance: The percentage of p16-positive cells in the endometrial stroma during the mid-luteal phase in the studied group of patients ranged between 0.03% and 6.73%, while it varied between 0.06% and 42.79% in the glands, and between 1.05% and 88.90% in the luminal epithelium. CD56-positive uNK cells were found only in the stroma and their abundance ranged between 0.01% and 34.17%.

No correlation was found between the frequencies of occurrence of senescent p16+ glandular and luminal epithelial cells and CD56+ cells ($p>0.05$). In contrast, the abundance of p16+ stromal cells showed low, but a significant correlation with the percentage of CD56+ stromal cells ($R=0.28$; $p<0.01$).
Limitations, reasons for caution: The study was limited in sample size.
Wider implications of the findings: Increased cell senescence in endometrial stroma during the mid-luteal phase is associated with rise in uNK cells in women with recurrent pregnancy failure. These results confirm the role of uNK cells in the selective clearance of senescent cells in the endometrium during the period of embryo implantation.
Trial registration number: NA

P-394 Aspirin and low-molecular-weight heparin combination therapy provides favorable immunomcompetent milieu to protect pregnancy in hyperhomocysteinemic women with recurrent pregnancy loss

P. Chakraborty[1], S. Chatterjee[2], D. Patra[2], S. Parvin[1], S. Kalapahar[1], R. Chattopadhyay[1], S. Ghosh[1], M. Chakraborty[1], S.N. Kabir[1], B. Chakravarty[1]

[1]Institute of Reproductive Medicine- Kolkata., Department of Assisted Reproduction, Kolkata, India ;
[2]Vidyasagar University, Tissue culture and molecular biology, Kolkata, India

Study question: Does aspirin and low-molecular-weight heparin (LMWH) combination therapy offer a favorable immunological milieu to salvage pregnancy in hyperhomocysteinemic women with recurrent pregnancy loss (RPL)?
Summary answer: Aspirin-LMWH combination therapy attenuates Th17 cell response and stimulates Th2-associated cytokine and chemokine production to confer immunocompetency and protect pregnancy in hyperhomocysteinemic women with RPL.
What is known already: The fetal immune tolerance is effected by a balance between T-helper (Th)-1, Th2, Th17, and regulatory responses involving both innate and adaptive immune cells orchestrated by signaling molecules (chemokines and cytokines). Women with RPL and/or recurrent implantation failure are reported with an elevated Th1/Th2 cell ratio and increased immune inflammatory responses at maternal-fetal interface. We have previously demonstrated that combined aspirin-LMWH anticoagulant therapy improves rate of pregnancy salvage in hyperhomocysteinemic women with RPL *(PLOS One 2013)*. We hypothesized that LMWH might persuade an immune-modulatory milieu, which could balance the pro-inflammatory response implicated in pathogenesis of RPL.
Study design, size, duration: This prospective cohort study in 53 hyperhomocysteinemic (homocysteine>12mmol/L) women with RPL is conducted between August 2017 and December 2019 at Institute of Reproductive Medicine, Kolkata. Patients (n=24) were supplemented with aspirin (5mg/day) alone or in combination with LMWH (2500IU/day) (n=29) for 12 weeks. They are analyzed for cytokines related to Th1 (interferon (IFN)-g, tumor-necrosis-factor (TNF)-a), Th2 (interleukin (IL)-10, IL-13) and Th-17 (IL-6, IL-23, transforming-growth-factor (TGF)-b) with their respective chemokines (CXCL10, CXCL11), (CCL17, CCL22) and (CCL20,CXCL1,CXCL8).
Participants/materials, setting, methods: Plasma samples obtained at gestational weeks (g.w.) 6 and 12 are analyzed by multiplex fluorochrome and Luminex performance assay for levels of Th1, Th17, and Th2-related cytokines and chemokines respectively. Percentage of Th17 and T-regulatory (Treg) cells in peripheral-blood-mononuclear-cells (PBMC) is detected by flow-cytometry. Differences in chemokine levels in between the groups were assessed by linear mixed models. Statistical significance was set at $p < 0.05$ as evaluated by student's T-test.
Main results and the role of chance: No differences are observed in cytokine and/or chemokine level/s at g.w. 6 between the groups. The percentage of Th17 cells (IL-17+/CD4+) significantly decreased ($2.98 \pm 0.68\%$ vs. $1.74 \pm 0.29\%$; $p<0.01$) with a simultaneous increase ($4.94 \pm 1.38\%$ vs. $3.03 \pm 0.91\%$; $p<0.02$) in percentage of Tregs (CD4+CD25brightFoxp3+ T cells) in aspirin-LMWH-treated cohort. The combination group, although documented a decreased concentration/s (pg/ml) of Th17-type cytokines (IL-6 (1.88 ± 0.65 vs. 0.91 ± 0.43; $p<0.04$ and IL-23(17.94 ± 9.76 vs. 7.78 ± 3.43; $p<0.01$)), at g.w. 12, concentration of TGF-β was significantly higher (3909.05 ± 1248.35 vs. 2469.83 ± 1058.71; $p<0.01$) which cue to probable maintenance of balance of inflammatory response in first trimester. Improvement in IL-10 concentration (6.32 ± 2.46 vs. 8.87 ± 4.32; $p<0.02$) was documented

after combination treatment. No differences were observed in Th1-type cytokine level/s (IFN-γ (1.97 ± 0.69 vs. 1.76 ± 0.34) and TNF-α (1.03 ± 0.37 vs. 0.98)) providing a favorable shift in the combination cohort indicating a Th2 bias. Mixed linear model test showed an increase in CCL22 ($p<0.05$); and a decrease ($p < 0.04$) in CCL20 during g.w.12 while CXCL10, CXCL11, CXCL1, CXCL8, CCL17, CCL22 do not differ during the treatment.

Limitations, reasons for caution: Future studies with higher sample size should corroborate the current findings. Moreover, this is an observational study not supported by appropriate/untreated controls because of stringent regulation/s from local ethics board. LMWH treatment was not continued beyond 12 weeks of gestation for absence of systematic network monitoring in our set-up.

Wider implications of the findings: Aspirin-LMWH supplementation augments the number of Treg cells and increases IL-10 that perhaps provide a favorable immunocompetent milieu to protect pregnancy in hyperhomocysteinemic women with RPL. Further ongoing studies will look into the duration of LMWH regimen for better immunosupression.

Trial registration number: NA

P-395 Evaluating the clinical utility of endometrial receptivity analysis test in women with recurrent pregnancy loss

M. Luna[1], T. Alkon[1], C. Hernandez-Nieto[1], D. Cassis-Bendeck[1], B. Sandler[1]

[1]Reproductive Medicine Associates of New York, Reproductive Endocrinology and Infertility, New York- NY, U.S.A.

Study question: Does endometrial receptivity analysis (ERA) test offer clinical utility in patients with recurrent pregnancy loss (RPL)?

Summary answer: Women with RPL may benefit from a personalized adjustment in timing of frozen embryo transfer.

What is known already: Successful embryo implantation requires an appropriate embryonic development, coincident with a receptive endometrium. Defects in endometrial receptivity contributing to implantation failure, remain not only the major rate-limiting step in vitro fertilization (IVF) success, but also a major culprit in unexplained infertility and spontaneous abortions. Because of this, the ERA test was developed. ERA test enables the determination of a personalized window of implantation (WOI). However, clinical evidence is still scarce regarding the value of performing an endometrial receptivity test in women with RPL.

Study design, size, duration: Monocentric prospective study from June 2018 to April 2019. 10 women with at least 2 consecutive miscarriages under 10 weeks of gestation of unexplained origin after IVF conceptions, underwent an ERA test. Subsequently, a single frozen euploid embryo transfer (SET) with appropriate adjustment in timing according to the ERA test was performed.

Participants/materials, setting, methods: Women with RPL of unexplained origin, <40 years, history of a full-term pregnancy after a euploid embryo transfer, normal karyotypes, uterine cavity assessed by hysteroscopy and no identified thrombophilia's were included. Participants were administrated oral/transdermal estradiol on cycle day two for endometrial preparation. When the endometrial thickness was ≥ 7 mm/ triple line pattern, luteal support was begun using vaginal and intramuscular progesterone. Endometrial biopsy was performed upon completion of 5 days of progesterone.

Main results and the role of chance: 100% of the patients were found to have a non-receptive endometrium. Five were pre-receptive (24 hours), three early receptive (12 hours), and two late receptive (12 hours). After a personalized SET was performed, 70% (7/10) became pregnant. Of the 70% of the women that achieved a pregnancy, 1 patient had a miscarriage at week 10 and the remaining had a live birth.

Limitations, reasons for caution: This a pilot study performed to test the utility of ERA test in women with RPL. Due to the size of the study, the effects of more subtle covariates would not have been detected. Future randomized controlled trials are needed.

Wider implications of the findings: A displaced implantation window was found in all the patients analyzed with RPL. These findings suggest for the first time, that the WOI may change after a full-term pregnancy or surgical uterine manipulation. These results enable appropriate study design of future studies.

Trial registration number: Not Applicable

P-396 Multidisciplinary approach in repeated implantation failure patients' evaluation

S. Bordoli[1], M. Scarrone[1], F. Pasi[2], V. Canti[3], L. Quaranta[3], R. De Lorenzo[1], S. Cella[1], V.S. Vanni[4], E. Papaleo[3], P. Giardina[3], P. Rovere Querini[1]

[1]Vita-Salute San Raffaele University, Internal and Specialistic Medicine, Milan, Italy ; [2]IRCCS San Raffaele Hospital, Obstetrics and Gynecology, Milan, Italy ; [3]IRCCS San Raffaele Hospital, IVF unit, Milan, Italy ; [4]Vita-Salute San Raffaele University, IVF Unit, Milan, Italy

Study question: Do repeated implantation failure (RIF) patients benefit from medical multidisciplinary evaluation?

Summary answer: An internist evaluation from an immunological, endocrinological and hematologic point of view, associated with the gynaecological one in RIF patients can improve their reproductive prognosis.

What is known already: No consensus regarding the definition of RIF has been reached. A widely accepted definition is the failure of implantation of at least three good quality embryos, in at least three fresh or frozen cycles transferred in women aged <40 years. These women are usually evaluated only in the context of IVF units. Pathogenesis is still unclear and multifactorial (embryo factors, maternal/endometrial factors, gamete factors and ART techniques associated factors). Recurrent pregnancy loss (RPL) is instead well known to benefit from thorough medical investigations since many causes other than gynaecological ones have been proved to be associated to RPL.

Study design, size, duration: We performed a prospective observational study of patients referred to recurrent pregnancy loss and implantation failure outpatient clinic at San Raffaele Hospital (Milan, Italy) between March 2019 and March 2020 (still ongoing). The patients reproductive complaints were RIF (n=43) or RPL (n=110).

Participants/materials, setting, methods: We studied patients according to demographic data, past medical history (genetic diseases, hypertension, abnormal thyroid function, congenital or acquired thrombophilia, history of DVT or pulmonary embolism, autoimmune diseases), past gynecological and obstetric history. Laboratory test have been prescribed to investigate autoantibodies, inflammatory indexes or dysmetabolic conditions. In order to assess the relevance of the evaluation and management, we have longitudinally followed patients during fertility procedures after the first visit and collected pregnancy rates and outcomes.

Main results and the role of chance: When we compared the prevalence of abnormal findings in RIF and RPL we found no statistically significant ($p>0.5$) difference in the distribution, suggesting that the factors contributing to the poor reproductive outcomes in these two dramatically different groups of patients may be similar. In a significant proportion of RIF patients we detected positive antinuclear antibodies (32.5%) antithyroid antibodies (18.6%), prothrombin mutation (11.5%), hyperhomocysteinemia and positive antiphospholipid antibodies (6.9%). Moreover, after appropriate treatment and proper support, 15 patients underwent a further IVF cycle and n=8 (implantation rate 0.53) had a positive pregnancy test, compared to an implantation rate of 0.15 normally reported in RIF patients undergoing a subsequent embryo transfer.

Limitations, reasons for caution: The small number of patients assessed so far in our multidisciplinary clinic is the main limitation of this work. An increment of data collection and pregnancy rate and outcomes will be of interest.

Wider implications of the findings: The establishment of a multidisciplinary team comprising an infertility specialist flanked by an internist could improve assisted reproductive technology (ART) outcomes by detecting immunological/endocrinological problems that may play a role in both implantation failure pathogenesis and physiological pregnancy development.

Trial registration number: non applicable

P-397 Low iron stores are associated with Recurrent Pregnancy Loss but does not predict subsequent loss or ability to conceive

M. Georgsen[1], M.C. Krog[2], A.S. Korsholm[3], H.W. Hvidman[3], A.M. Kolte[1], A.S. Rigas[4], H. Ullum[4], S. Ziebe[3], A.N. Andersen[3], H.S. Nielsen[5], M.B. Hansen[6]

[1]Copenhagen University Hospital- Rigshospitalet, The Recurrent Pregnancy Loss Unit- The Fertility Clinic, Copenhagen, Denmark ;

[2]Copenhagen University Hospital- Rigshospitalet, The Recurrent Pregnancy Loss Unit- The Fertility Clinic and Department of Clinical Immunology, Copenhagen, Denmark ;
[3]Copenhagen University Hospital- Rigshospitalet, The Fertility Clinic, Copenhagen, Denmark ;
[4]Copenhagen University Hospital- Rigshospitalet, Department of Clinical Immunology, Copenhagen, Denmark ;
[5]University of Copenhagen- Rigshospitalet- and Amager-Hvidovre Hospital, The Recurrent Pregnancy Loss Unit and Department of Gynaecology-and-obstetrics, Copenhagen, Denmark ;
[6]Copenhagen University Hospital- Rigshospitalet, Department of Clinical Immunology, Copenhagen, Denmark

Study question: Is low iron stores associated with Recurrent Pregnancy Loss (RPL) and does low iron stores impair the ability to conceive?
Summary answer: S-ferritin levels were inversely related to number of previous losses in women with RPL. Low iron stores did not affect the ability to conceive.
What is known already: Iron requirements are increasing during pregnancy and iron deficiency is common among women of reproductive age. Iron is essential for hemoglobin production, cellular metabolism and the immune system. Iron deficiency has been suggested to affect fertility due to the iron requirements of the developing oocytes. Low maternal iron stores result in lower birth weight and increase the risk of preterm birth. Studies of iron stores and sporadic pregnancy loss are sparse and contradictory. Furthermore, an association between iron deficiency and RPL has not previously been studied.
Study design, size, duration: A cohort study comparing s-ferritin levels in women with RPL (n=84) referred between 2013-2016 with a control group of women of reproductive age with no known fertility problem (n=153). The study investigated whether s-ferritin correlated to the ability to conceive and time to conception during the first two years after sampling and whether s-ferritin were associated with outcome of the first pregnancy after referral for RPL women.
Participants/materials, setting, methods: The study included women with RPL (≥3 pregnancy losses) referred to the Danish RPL unit and women who consulted the Fertility Assessment and Counselling Clinic both located at Copenhagen University Hospital, Denmark. Pregnancy history were recorded and serum samples were collected at first consultation and stored at -20°C until analysed. Follow-up on verified pregnancies was done after two years for both groups. Women who did not try to conceive or underwent fertility treatment were excluded.
Main results and the role of chance: Women with RPL had lower s-ferritin compared with the control group, 39.9 µg/L versus 62.2 µg/L (p=0.001) and had a higher prevalence of low iron stores (s-ferritin <30 µg/L), 35.7 % versus 13.7 % (p<0.001). There was an inverse relationship between s-ferritin levels and number of pregnancy losses before referral for the RPL women. When divided into groups of 1) 2-3 losses, 2) 4 losses, and 3) ≥5 losses, median s-ferritin levels differed significantly between the groups (p=0.010). There was no association between s-ferritin levels and the ability to conceive or time to pregnancy in neither the RPL nor the control group. S-ferritin did not predict the risk of losing the first pregnancy after referral in the RPL group.
Limitations, reasons for caution: This study is limited by the relatively small sample size and the low prevalence of iron deficiency in the studied population which may explain why we did not find a correlation between iron deficiency and subsequent pregnancy loss or ability to conceive.
Wider implications of the findings: The findings indicate that low s-ferritin could be a relevant risk factor in women with RPL. It should be investigated whether iron status should be assessed routinely before pregnancy in both healthy women and women with RPL and whether iron supplementation may increase the chance of live birth.
Trial registration number: Not applicable

P-398 Does pregnancy implantation site and trophoblastic ring thickness at early gestational scan predict outcomes of an intrauterine pregnancy? An observational study

L. Nancarrow[1], S. Vinayagam[1], M. Swanson[1], K. Ford[1], D. Hapangama[2], R. Hombury[3], R. Russell[1]

[1]Liverpool Womens Hospital, Hewitt fertility centre, Liverpool, United Kingdom ;
[2]University of Liverpool, Institute of translational medicine, Liverpool, United Kingdom ;
[3]Homerton University Hospital, Homerton fertility centre, London, United Kingdom

Study question: Does the pregnancy implantation site and trophoblastic ring thickness in an early scan predict outcomes and complication rates of intrauterine pregnancies?
Summary answer: Pregnancies located in the lower half of the uterine cavity with a thinner trophoblastic ring were more likely to miscarry.
What is known already: Women routinely have early gestation scans to assess the location and to confirm viability of pregnancy. This reassures most women who continue to have an uneventful pregnancy, although unfortunately some will later have a miscarriage or later pregnancy complications. Trophoblastic ring may be related to placentation and no previous studies have examined the predictive role of trophoblastic ring thickness at time of early gestation scanning in pregnancy outcomes and late pregnancy complications. Implantation sites after an embryo transfer had not been associated with pregnancy outcomes.
Study design, size, duration: This was a prospective observational study in a tertiary referral publically funded IVF unit in UK. 300 patients who attended for early pregnancy scan following single embryo transfer over a 14-month period were included after obtaining Informed consent.
Participants/materials, setting, methods: Patient typically underwent their early pregnancy scan between 6-8 weeks gestation. Inclusion criteria included any live ongoing singleton pregnancy following single embryo transfer and a 3D image was obtained of the uterus. Exclusion criteria were Women with uterine cavity abnormalities, multiple pregnancies or double embryo transfers were excluded. Patients were followed up to until the end of pregnancy, either miscarriage, term or preterm birth. Any adverse pregnancy outcomes (pre-eclampsia, IUGR etc) were also recorded.
Main results and the role of chance: Of the 300 patients recruited, at this interval review 286 pregnancies were ongoing with the remaining 14 ending up as miscarriages. Women who miscarried were older than those with ongoing pregnancies (mean ages 36.3 vs 33.5 years; p=0.002) but there were no other demographic differences were identified (e.g. BMI). Mean trophoblastic ring was thicker in the ongoing pregnancy group when compared with women who miscarried (7.14mm vs. 5.4mm; p=0.008).

Of the 14 miscarriages, 4 were located in the lower half of the uterine cavity. 18 pregnancies were located in the lower half of the cavity in the remaining ongoing pregnancies. This indicated a significant increase in miscarriage rate when implantation occurred in the lower half of the cavity (p=0.01).
Limitations, reasons for caution: This is an observational study without intervention. The observed difference may be attributable to the differences in patient age, but the slight difference of <3 years is unlikely to be the sole reason.
Wider implications of the findings: Investigation of trophoblastic ring thickness as a marker of pregnancy outcome/complications is warranted. Identifying non-invasive biomarkers facilitating studies of trophoblast invasion could lead to development of therapeutic strategies targeting complications of pregnancy. Avoiding embryo deposition in the lower cavity and correlating implantation site following embryo transfer also requires further investigation.
Trial registration number: not applicable

P-399 Proteomic signatures in Assisted Reproduction Technology (ART) cycles

V. Kanaka[1], A. Papadopoulou[2], S. Proikakis[2], P. Drakakis[1], T.G. Tsangaris[1], D. Loutradis[1]

[1]Department of Obstetrics and Gynecology, Alexandra Hospital, School of Medicine ;
[2]Proteomics Research Unit, Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Study question: The identification of biomarkers for successful embryo implantation and pregnancy onset in In Vitro Fertilization (IVF) by proteomic analysis
Summary answer: Twenty four proteins were differentially expressed among women achieving and not achieving pregnancy after IVF and embryo implantation
What is known already: More than 400,000 babies from 1,6 million Assisted Reproduction Technology (ART) cycles are born around the world every year. The efficacy of in vitro fertilization cycles (IVF) has improved significantly over the past 40 years. However better understanding of the reproductive process may help increase birth rate. Proteomic analysis of the embryo secretome that

has already been done has shown a positive correlation between high levels of leptin and human leukocyte antigen (HLA) with the implantation potential of the embryo. Moreover previous studies have shown that protein AMBP and Leucine-rich alpha-2-glycoprotein play an important role in the implantation process.

Study design, size, duration: Proteomic analysis was performed for the analysis of peripheral blood serum of women undergoing IVF treatment age 30-43. Blood was collected prior to the IVF program, at the day of embryo transfer as well as 6 and 12 days post embryo transfer. In order to compare successful and unsuccessful cycles, twenty women is the size of the study by which ten has successful implantation and ten unsuccessful in a three years duration of the study.

Participants/materials, setting, methods: After serum was obtained, two dimensional gel electrophoresis (2DE) followed by MALDI-TOF–MS/MS Mass Spectrometry were performed to analyze the differential expression of proteins in the serum samples among women having successful and unsuccessful IVF cycles.

Main results and the role of chance: Twenty four proteins were differentially expressed among women achieving and not achieving pregnancy. Specifically, protein AMBP, Apolipoprotein A-IV, Growth/differentiation factor 2, Glucosamine-6-phosphate isomerase 2, G patch domain and ankyrin repeat-containing protein 1, Hemoglobin subunit beta, Kelch-like protein 23, Zinc-alpha-2-glycoprotein, Leucine-rich alpha-2-glycoprotein, Vitronectin, protein NATD1 and Plasminogen were expressed only in successful cycles. Proteins that were present only in unsuccessful resulting cycles are proteins that belong mainly to the immune system and include Complement C1r subcomponent, Complement C1s subcomponent, Complement C1q subcomponent subunit B, Ig mu heavy chain disease protein, Ig mu chain C region as well as Hemopexin, Serum paraoxonase/arylesterase 1, Alpha-2-macroglobulin, Tyrosine-protein kinase STYK1, Angiotensinogen, Keratin, type I cytoskeletal 9 and Keratin, type II cytoskeletal 1

Limitations, reasons for caution:

–No Donor cycles

–Age limited to 30-43 years old

–The results must be evaluated in a larger cohort and longer duration of time.

Wider implications of the findings: Predicting pregnancy and improving drugs for IVF treatment. Improve IVF pregnancy rates and success.

Trial registration number: non applicable

P-400 Ovarian hyperstimulation and high levels of progesterone are detrimental for ectopic pregnancy in ART

I. Oprova[1], N. Magunska[2], T. Milachich[3], A. Shterev[2], P. Andreeva[1]

[1]Shterev Hospital, Assisted Reproduction Department, Sofia, Bulgaria ;
[2]Shterev Hospital, Department of Obstetrics and Gynecology, Sofia, Bulgaria ;
[3]Shterev Hospital, IVF and Andrology Laboratory, Sofia, Bulgaria

Study question: Could ovarian hyperstimulation and high levels of Progesterone be a risk factor for an ectopic pregnancy in ART?

Summary answer: Endometrium thickness over 12 mm and progesterone level under 1 ng/ml and FrET could be a preventive measure for EP development.

What is known already: The ectopic pregnancy (EP) rate has been reported higher after assisted reproductive technology (ART) than after a spontaneous conception. The factors associated with abnormal implantation are widely discussed and not completely clear.

Study design, size, duration: The present study was an observational retrospective trial, performed in a private IVF clinic. It encompassed 366 women who have undergone in vitro fertilization treatment: stimulated cycles with fresh embryo transfers (STIM ET) and hormone replacement therapy with frozen embryo transfers (Fr ET).

Participants/materials, setting, methods: Four features were evaluated: endometrial thickness at the day of ET, stage of the embryos transferred – cleavage stage (day 3) or blastocyst stage (day 5), the progesterone level on the day of hCG in stimulated cycles, ovarian hyperstimulation (OHS). The primary outcome measure was a development of EP. Logistic regression was performed to utilize the significance of the numbers.

Main results and the role of chance: At total of 366 Fresh ET or Fr ET cycles were compared, 41 with EP and 325 with an intrauterine pregnancy. We detected that thin endometrium could be risky for developing EP, but only in stimulated cycles (endometrium in STIM ET and EP = 10.7 mm versus STIM ET and no EP = 11.8 mm; p = 0.029). In contrast, with Fr ET cycles, the size of the lining was not important for the occurrence of EP (Fr ET and EP = 10.6 mm versus Fr ET and No EP = 11.14 mm; p = 0.2). This comparison emphasizes the observation that EP is more often detected in stimulated than in frozen cycles (61.98 % versus 39.02%; p = 0.04). Something more, most of the EP are resulted after a transfer of blastocyst stage embryos but only in stimulated cycles (EP rate 52% versus 21%, p = 0.0009). Also, we have established that high level of Progesterone on the day of hCG trigger could contribute for an ectopic embryo implantation (progesterone in STIM ET and EP = 1.05 ng/ml versus STIM ET and no EP = 0.59 ng/ml; p = 0.0014).

Limitations, reasons for caution: The study was a retrospective cohort study at a single center, larger trials with an increased number of patients needed to confirm our finings.

Wider implications of the findings: This study shows an increased risk of an ectopic pregnancy in stimulated ET cycles than in FrET. These findings suggest elevated progesterone and hormonal milieu of ovarian stimulation was detrimental for ectopic pregnancy. Freeze all strategy followed by Fr ET would decrease chance of ectopic pregnancies in ART.

Trial registration number: Not applicable

P-401 Characterization of bHCG trends in Euploid Frozen-Thawed Embryo Transfer Cycles by Initial Value

J. Chervenak[1], R. Setton[2], A. Petrini[2], K. Hancock[2], P. Chung[2], Z. Rosenwaks[2]

[1]New York Presbyterian/Weill Cornell, Obstetrics and Gynecology, New York, U.S.A. ;
[2]Center for Reproductive Medicine, Obstetrics and Gynecology, New York, U.S.A.

Study question: What is the predictive value of initial bHCG change stratified by initial bHCG value in frozen-thawed embryo transfer (FET) cycles with preimplantation genetic testing (PGT)?

Summary answer: This study affirms that the initial bHCG level and its initial rate of change are strong independent predictors of live birth in euploid FET.

What is known already: Existing studies have established initial bHCG level and subsequent rate of rise after IVF as one of the earliest means of predicting pregnancy outcome. Over the past decade, the proportion of FET and PGT have increased. There is limited data to inform prognostic counseling in this population. Further, the effect of the initial value of bHCG on the expected rate of rise of bHCG has not been well characterized.

Study design, size, duration: This is a retrospective cohort study in which all FET cycles that had a transfer of a single PGT-euploid embryo performed in an academic medical center from 2013 to 2019 were screened for inclusion. Only cycles that resulted in a positive initial serum bHCG on CD 28 or 29, followed by a rise in the serum bHCG value 48 hours later, and a subsequent maximum of one gestational sac were included in the study.

Participants/materials, setting, methods: There were 596 FETs during the study time period that met the inclusion criteria. Groups were stratified by initial bHCG level (group 1: <50 mIU/mL; group 2: ≥50 mIU/mL and ≤100; and group 3: >100 mIU/mL) and compared across the rate of change of this value over two days (≤50%, 51-99%, and ≥100%). Statistical analysis was performed using Chi-square and Fisher's exact t-test.

Main results and the role of chance: Baseline age and BMI were similar for all groups. For group 1, the live birth rate (LBR) was the lowest of the cohorts (LBR 19.57%) and there was no significant difference in the chance of live birth regardless of whether bHCG rose <50% (LBR 10%), between 51-99% (LBR 10%), or ≥100% (LBR 26.9%, p=0.16). For group 2, the LBR significantly increased across the three categories of rate of bHCG rise (LBR 25% for ≤50% rise, vs. 54.5% for 51-99% rise, vs. 70.5% for ≥100% rise, p<0.05). Similarly for group 3, the LBR significantly increased across the three categories (LBR 10% for ≤50% rise, vs. 84.1% for 51-99% rise, vs. 90.1% for ≥100% rise, p<0.05 for all). For groups 2 and 3, an increase of bHCG of more than 50% is strongly associated with an increased LBR (p=0.02 and p=<0.0001, respectively). In the absence of a bHCG rise of >50% over 2 days, there was no significant difference noted between the 3 groups and prognosis was poor (LBR 14.29%).

Limitations, reasons for caution: This study examines bHCG values taken during a narrow interval of days after FET. The results of this study may not be generalizable to values taken outside of this timeframe.

Wider implications of the findings: The findings of this study elucidate the relative impact of initial serum bHCG values in FET with PGT cycles for predicting live birth and offer a counseling tool for the clinician.
Trial registration number: not applicable

P-402 The impact of chronic endometritis on pregnancy outcomes in women with recurrent implantation failure

Y. Liu[1], X. Chen[1], W.C. Cheung[1], Y. Zhao[1], R. Qi[1], J.P.W. Chung[1], C.C. Wang[1], T.C. Li[1]

[1]The Chinese University of Hong Kong, Department of Obstetrics and Gynaecology, Shatin- Hong Kong, China

Study question: Does the presence of chronic endometritis (CE) affect pregnancy outcomes in women with recurrent implantation failure (RIF)?
Summary answer: Chronic endometritis was associated with a lower successful implantation rate in women with RIF.
What is known already: Chronic endometritis is a state of persistent inflammation in the endometrial lining, which has been reported to be associated with reproductive failure, including RIF. Plasma cell density in the entire endometrial biopsied tissue has been put forward as a more reliable and accurate method of plasma cell assessment for the diagnosis of CE, and that the prevalence of CE in women with reproductive failure was only 10%. However, the proof of this measurement requires clinical studies to confirm that it is of useful prognostic value and leads to effective treatment on CE.
Study design, size, duration: This is a case-control observational study on pregnancy outcomes in 64 reproductive-age women with RIF undergoing endometrial scratch in a natural cycle preceding frozen-thawed embryo transfer with the use of nondonor oocytes. All the women are classified with CE status using a stringent plasma cell density threshold, > 5.15 CD138+ plasma cells/10 mm^2, which was the 95th percentile of a fertile control population and served to establish a normal reference range.
Participants/materials, setting, methods: Women failing to achieve a clinical pregnancy after transfer of at least four good-quality embryos in three or more transfer cycles and seeking treatment in our unit were included. Endometrial specimens were collected precisely 7 days after LH surge (LH+7) and plasma cell density was determined based on Syndecan-1 (CD138) positive cells in the entire biopsy section. The clinical information (e.g. successful implantation rate and clinical pregnancy rate) for these patients was reviewed and analyzed.
Main results and the role of chance: Among 64 patients recruited, 24 of them were excluded because of the loss of follow-up and high uterine killer cells count. Thus, a total of 40 patients were studied. 10% (4/40) of them were found to have CE, and the other 90% (36/40) did not have CE. There was no difference in maternal age, body mass index, history of pregnancy or duration of infertility between the CE and non-CE groups.

We observed a lower successful implantation rate in the CE group when compared with the non-CE group [50.0% (2/4) *vs.* 52.8% (19/36), respectively], while the unsuccessful implantation rate is higher in the CE group [47.2% (17/36)] than the non-CE group [50.0% (2/4)], but without a significant statistical difference (p =1.000).

However, the clinical pregnancy rate is higher in CE group than non-CE group [50% (2/4) *vs.* 36.1% (13/36), respectively]. Further, the non-pregnancy rate is lower in CE group [50.0% (2/4) *vs.* 63.9% (23/36), respectively, p =0.622].
Limitations, reasons for caution: The limitation of this study is that it only involved four women with CE due to its prevalence of 10% in our unit using the novel diagnostic method, which precludes the analysis of the relationship between CE and pregnancy outcomes in women with RIF.
Wider implications of the findings: We reported the presence of CE may affect the successful implantation rate in women with RIF. We reason that CE may provide a role in predicting the prognostic value in other subgroups of reproductive failure, such as recurrent miscarriage and infertility.
Trial registration number: ChiCTR-IOC-16007882

P-403 Shortened time to live birth in subfertile women undergoing endometrial diagnostic biopsy

V. Mitter[1], S. Meier[2], T.T. Rau[3], M. Von Wolff[1], M. Zwahlen[4], A.S. Kohl Schwartz[3]

[1]University Women's Hospital and University of Bern, Gynaecological Endocrinology and Reproductive Medicine, Bern, Switzerland ;
[2]University Women's Hospital, Gynaecological Endocrinology and Reproductive Medicine, Bern, Switzerland ;
[3]Bern University Hospital and University of Bern, Institute of Pathology, Bern, Switzerland ;
[4]University of Bern, Institute of Social and Preventive Medicine, Bern, Switzerland

Study question: What is the impact of diagnostic endometrial biopsy and treatment of chronic endometritis (CE) on the time-to-pregnancy and live birth in women with recurrent pregnancy loss or recurrent implantation failure?
Summary answer: Diagnostic endometrial biopsy and treatment of chronic endometritis reduces time-to-pregnancy an time to live birth in women with recurrent pregnancy loss or recurrent implantation failure.
What is known already: Up to 57% of women with recurrent pregnancy loss (RPL) and up to 66% of women with recurrent implantation failure (RIF) suffer from CE. Recently, a meta-analysis showed higher pregnancy and live birth rates after antibiotic treatment for CE in women with RIF. This was also found in a meta-analysis of trials on endometrial scratch injury in women who had two or more implantation failures. However, there remains a need to investigate the effect of diagnosis and treatment of CE and endometrial scratch injury specifically in women with RPL and RIF.
Study design, size, duration: This is a retrospective analysis of a cohort of women at the University Women's Hospital of Bern, Switzerland diagnosed for recurrent pregnancy loss or recurrent implantation failure between January 2014 and April 2019 (n=127). 108 fulfilled inclusion criteria, 61 with RPL and 47 with RIF. 41 served as historical controls as we only performed hysteroscopic assessment without biopsy and detection of plasma cells until 2016.
Participants/materials, setting, methods: We defined RPL as ≥ three consecutive miscarriages and RIF as ≥ six toP-quality cleavage-stage embryos unsuccessfully transferred. We performed diagnostic hysteroscopy and endometrial biopsy. The pathologist stained endometrial tissue with hemalaun-eosin and immunohistochemically for CD138. In cases of chronic endometritis, defined as presence of ≥ one plasma cell, we treated with antibiotics. We used Cox regression to adjust for confounding factors such as age of mother and parity and stratified by RPL and RIF.
Main results and the role of chance: Of 108 women, forty-one (38%) had standard hysteroscopic assessment and served as control (control group). The remaining 67 (62%) women had a diagnostic endometrial biopsy and histopathological assessment. In 25 (37%) women, no plasma cells were detected (CEneg). Forty-two (63%) women were positive for chronic endometritis and subsequently treated with doxycycline 100mg twice a day orally for two weeks (CEpos). The women in CEpos group (hazard ratio = 2.86; 95% CI 1.45 – 5.63; p= 0.002) had a higher, and women in CEneg group (HR =2.11; 95% CI 0.87 – 5.08; p=0.095) a possibly higher chance for a clinical pregnancy than women did in the control group. We observed similar results for the probability of a live birth: for women of CEpos group, the hazard ratio is 4.00 (95% CI 1.65 – 9.71; p=0.002) and for women of CEneg the hazard ratio is 2.02 (95% CI 0.71 – 5.70; p=0.184), both compared to the control group. In all women with biopsy (groups CEng and CEpos), the hazard ratio was 2.62 (CI 95% 1.39 – 4.94; p=0.003) for a clinical pregnancy and 3.00 (CI 95% 1.38 – 6.52; p= 0.005) for a live birth compared to the control group.
Limitations, reasons for caution: This is a retrospective analysis of data from our center. We did not perform test-of-cure after Treatment unless a woman had a subsequent miscarriage. However, our interest lied in the general impact on the time-to-pregnancy of an endometrial diagnostic biopsy and subsequent Treatment of CE.
Wider implications of the findings: In women suffering from RPL or RIF, we suggest performing endometrial biopsy, diagnosis and treatment of CE to shorten the time-to-pregnancy and live birth. Endometrial biopsy itself might have an effect on time-to-pregnancy and live birth. Possible rehabilitative healing processes may need some time.
Trial registration number: not applicable

POSTER VIEWING SESSION
MALE AND FEMALE FERTILITY PRESERVATION

P-404 The impact of the BRCA gene mutations on the reproductive potential in patients with active breast cancer undergoing fertility preservation

S. Arab[1], A. Badeghiesh[2], W. Buckett[3]

[1]McGill University, Department of Obstetrics and Gynecology-Reproductive Endocrinology and Infertility Centre, Montreal, Canada ; [2]McGill University, Department of Obstetrics and Gynecology, Montreal, Canada ; [3]McGill University, Department of Obstetrics and Gynecology-Reproductive Endocrinology and Infertility Center, Montreal, Canada

Study question: Do breast cancer patients with the BRCA gene mutations have lower ovarian reserve and poorer ovarian response than non-BRCA breast cancer patients?
Summary answer: Breast cancer patients with the BRCA gene mutations have similar ovarian reserve and ovarian stimulation respond compared to non-BRCA breast cancer patients.
What is known already: Controversy exists about fertility and ovarian reserve in BRCA mutation carriers. Some studies suggest that these patients may have low ovarian reserve and poor ovarian stimulation response. However, most data exists in carriers without breast cancer, rather than women with breast cancer undergoing fertility preservation prior to gonadotoxic treatment. What data does exist in breast cancer patients is from very small studies with conflicting results. Concerns about poorer response to stimulation in BRCA carriers may lead to women electing not to pursue fertility preservation or to being overstimulated.
Study design, size, duration: A retrospective cohort study of 131 breast cancer patients with known BRCA status who had had undergone fertility preservation at a university teaching hospital ART center from 2005-2019
Participants/materials, setting, methods: Breast cancer patients < 40 years old who had undergone fertility preservation with either IVF or IVM before starting gonadotoxic therapy; known BRCA status; both ovaries were present and no ovarian disease or surgery. Before starting the treatment, antral follicle count (AFC) was determined. Total of oocytes retrieved, number of MII oocytes , oocyte fertilization rate (where appropriate) and the number of oocytes and/or embryos cryopreserved were compared according to BRCA status.
Main results and the role of chance: Of 244 breast cancer patients <40 years who underwent fertility preservation between 2005 and 2019, 131 had known BRCA status and 125 fulfilled the above criteria. A total of 94 (75%) patients were BRCA negative, of whom 52 (55%) underwent fertility preservation with stimulation (IVF) and 42 (45%) without stimulation (IVM), and 31 (25%) patients were BRCA positive, of whom 16 (52%) underwent IVF and 14 (45%) underwent IVM .The patient mean ages (30+/-4.1 vs 32+/-4.2)(P=0.98), AFC (17+/-10 vs 17+/-9) (P=0.83) and, where appropriate, total gonadotrophin dose (2468 IU vs 1961 IU)(P=0.46) and days of stimulation (8.7+/-62 days vs 7.2+/-5.4 days)(P=0.26) were similar in BRCA positive compared to BRCA negative patients. In terms of reproductive response, there were no significant different between BRCA positive and BRCA negative patients in the total number of eggs retrieved overall (12.1+/-9 vs 11.0+/-8)(P=0.65), the total number eggs retrieved following IVF (14.1+/-7.2 vs 12.7+/-6.1)(P=0.47) or IVM (11.9+/-10.6 vs 10.4+/-9.2)(P=0.60), the number of MII oocytes collected (5.5+/-6.5 vs 5.2+/-5.1) (P=0.76), the number of fertilized oocytes (where appropriate) (4.9+/-6.0 vs 3.2+/-4.9)(P=0.18), the number of cryopreserved oocytes (3.1+/- 3.6 vs 2.0+/-2.9)(P=0.16)and the number of cryopreserved embryos (4.2+/-7.1 vs 4.8+/-6.5)(P=0.37). There was no difference between BRCA1 and BRCA2
Limitations, reasons for caution: The main limitations are the retrospective cohort study design which could introduce unidentified biases (although the BRCA status was not known beforehand in most cases) and the relatively small sample size which would miss small but real differences.
Wider implications of the findings: Comparable ovarian reserve and reproductive performance when undergoing fertility preservation should be reassuring for BRCA mutation carriers with breast cancer that outcomes are similar to non-BRCA carriers. BRCA carriers with breast cancer should be offered fertility preservation and protocols should not be changed solely on the basis of BRCA status.
Trial registration number: MUHC (ARSU6002/2020-6219)

P-405 Low in vitro maturation rates of oocytes recovered during ovarian tissue cryopreservation of very young girls compared to post-menarche patients undergoing fertility preservation before oncotherapy

Y. Fouks[1], E. Hamilton[2], Y. Cohen[1], Y. Hasson[1], Y. Kalma[1], F. Azem[1]

[1]Tel-Aviv Medical Center affiliated to the Sackler Faculty of Medicine- Tel-Aviv University, Lis Maternity Hospital- Department of Obstetrics and Gynecology-Fertility Division-, Tel Aviv, Israel ; [2]Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv, Israel

Study question: What are the outcomes of IVM of oocytes in very young patients scheduled for onco-therapy?
Summary answer: Oocytes recovered from girls younger than 5 years of age who undergo fertility preservation have a lower maturation rate in IVM compared to older females.
What is known already: In vitro maturation (IVM) of oocytes recovered during ovarian tissue cryopreservation is often practiced although it is still considered experimental. To date, only a few studies have examined the success of this maturation process in pre-menarchal girls.
Study design, size, duration: A total of 93 patients aged 0–25 years who underwent ovarian tissue cryopreservation as part of onco-fertility preservation between 2007 and 2019 were included in the study.
Participants/materials, setting, methods: Oocytes were recovered from the medium following ovarian tissue cryopreservation protocol and matured over 48 hours. Their development and maturation rate were recorded and compared between the different age groups.
Main results and the role of chance: The patient's age was positively and inversely correlated with the total number of mature oocytes. The absolute maturation rate in post-menarche and pre-menarche patients differed significantly (32.7% vs 11.3%, $P > 0.001$, respectively), while the degeneration rate of the oocytes did not (38.9% vs 33.4%, $P = 0.167$). The pre-menarche group had significantly lower rates of total mature oocytes compared to the post-menarche group (99 vs 9, $P = 0.004$). A subanalysis of the oocytes recovered from patients aged 1-5 years demonstrated that very few mature oocytes completed the IVM protocol.
Limitations, reasons for caution: This study was limited by its small sample size and retrospective nature.
Wider implications of the findings: Oocytes recovered from girls younger than 5 years of age who undergo fertility preservation have a lower maturation rate in IVM compared to older females. This may indicate a need for alternative methods for preserving the oocytes of very young patients.
Trial registration number: N/A

P-406 Pregnancy outcomes and affecting factors in the patients undergoing fertility treatments after high-dose medroxyprogesterone acetate therapy for endometrial cancer

A. Kawasaki[1], Y. Mori[1], H. Itagaki[1], H. Ijiri[1], T. Sato[1]

[1]University of Tsukuba, Department of Obstetrics and Gycecology, Tsukuba, Japan

Study question: Are fertility treatments beneficial for the patients after conservative management of endometrial cancer? What are affecting factors for the success of fertility treatments?
Summary answer: Fertility treatments bring good pregnancy outcomes for the patients after high-dose medroxyprogesterone acetate (MPA) therapy for endometrial cancer. Several factors may reduce pregnancy outcomes.
What is known already: MPA therapy is an option for young patients in an early stage of endometrial cancer who wish to preserve their fertility. While fertility treatments can lead to early pregnancy and bring relatively good pregnancy outcome for patients after MPA therapy, we must pay attention to disease recurrence. Some affecting factors for pregnancy outcomes such as endometrium damage, tumor characters etc. are indicated but enough evidence has not been shown.
Study design, size, duration: In this study, 40 patients of endometrial cancer after MPA therapy are recruited from the database of our institute from 1998 to 2018. In those patients, 22 patients underwent fertility treatments just after getting remission of endometrial cancer (FT group). Age-matched control groups were made of 213 general infertility patients who underwent reproductive treatments (C group) and 127 patients who underwent IVF-ET in our institute(C-IVF group).

Participants/materials, setting, methods: Clinical pregnancy rate and live birth rate were compared between FT group and C group or C-IVF group. We also calculated recurrence rate and compared between FT group and a group of patients who underwent no fertility treatments after MPA therapy (non-FT group). Next, we investigated the affecting factors on pregnancy outcomes of patients in FT group. The data were analyzed using t-test and chi-square test. The level of significance was set at $P<0.05$.
Main results and the role of chance: The pregnancy rate in FT group was 59.1% while 56.8% in C group. The pregnancy rate was 44.9% in C-IVF group and 35.7% in patients who underwent IVF-ET in FT group. No statistically significant difference was observed in the pregnancy rate between FT group and control groups. The disease recurrence rate was 31.6% in FT group and 21.4% in non-FT group. Regarding patients who failed to get pregnancy in FT group, thin endometrium, low ovarian reserve, changing institute for fertility treatments and disease recurrence were common factors related to the failure.
Limitations, reasons for caution: To confirm the results of this study, the sampling numbers are too small. A multi-center study is desirable to collecting more data. The large scale analysis is necessary to rule out cofounding factors and prove true variables affecting pregnancy outcomes.
Wider implications of the findings: Patients undergoing infertility treatments after MPA therapy should pay attention to the possibility of increasing risk of disease recurrence. The adverse factors are considered in performing fertility treatments. A close relationship between specialists of oncology and reproduction is important not to miss disease recurrence.
Trial registration number: not applicable

P-407 Fifteen year single center experience in sperm banking for cancer patients: use and reproductive outcomes in survivors

S. Stigliani[1], C. De Leo[2], E. Maccarini[1], P. Scaruffi[1], I. Casciano[1], P. Anserini[1]

[1]IRCCS Ospedale Policlinico San Martino, UOS Physiopathology of Human Reproduction, Genoa, Italy ;
[2]University of Genoa, DINOGMi, Genoa, Italy

Study question: Which are usage rates of frozen semen and results of in vitro fertilization (IVF) cycles in cancer patients that cryopreserved their semen before gonadotoxic treatments?
Summary answer: The percentage of patients who used their frozen semen was 6% and the outcomes of the IVF cycles confirmed the validity of the procedure.
What is known already: Some cancer diagnoses and treatments can place patients at risk for infertility, since gonadotoxic treatments may damage spermatogenesis. Sperm cryopreservation is an established and safe technique recommended by international guidelines for cancer-related fertility issues, to preserve fertility of cancer patients who are treated during their reproductive years. Literature data report not uniform rates - ranging from 5% to 29% - of cancer survivors' use of their cryopreserved semen. Collection of follow-up data about reproductive outcomes of cancer survivors who use their frozen semen in IVF cycles is necessary to evaluate the clinical impact of this procedure.
Study design, size, duration: This was a retrospective study which reported data of 683 cancer patients who referred to our center from 2004 to 2019 for fertility counseling and preservation before gonadotoxic therapies.
Participants/materials, setting, methods: A total of 632 oncological patients cryopreserved their own semen following international guidelines. Semen was evaluated according to the World Health Organization 2010 manual and it was cryopreserved using standard slow freezing method with nitrogen steam. The ICSI cycles were performed according to conventional procedures and outcomes were collected.
Main results and the role of chance: Among the 683 cancer patients who referred to our laboratory, the most frequent pathologies were leukemia and lymphoma (44%), followed by testicular cancer (35%). Patients' median age was 32 years (range 13-65). A total of 632 patients cryopreserved their semen. Over the years, 46% of patients continued to renew maintenance of their cryopreserved samples; 32% of patients were lost at follow-up; 10% were discarded according to patient's will; 6% of banked samples were discarded because patients died; 2% of patients moved their sperm samples to another fertility center for IVF treatments; 4% used their frozen semen for IVF at our center. Men who used their frozen semen for IVF cycles were survivors of leukemia and lymphomas (52%), and testicular cancer (20%) and others (28%). At the time of cryopreservation patients were in childbearing age (median age: 35 years, range 21-53) and at the time of IVF treatments the median age was 38 years (range: 26-54). As regard as outcomes, 33 ICSI cycles were performed with the frozen sperms, fertilization rate was 68%, implantation rate was 25%, cumulative birth rate was 30%, with a total of 15 healthy babies born from 2009 to 2019.
Limitations, reasons for caution: This was a single-center study reporting data from a limited number of patients. Since our center is the regional referent semen bank, we will continue on banking semen of oncological patients and on collecting follow-up data. Thus, clinical value and limitations will be deepened in a larger cohort.
Wider implications of the findings: Sperm cryostorage is simple and feasible for male adults and adolescents and it should be part of fertility management for patients candidate for receiving gonadotoxic therapies. The usage rate may increase during the years and a regular follow-up may prevent storage of unnecessary samples.
Trial registration number: not applicable

P-408 Clinical evidence of follicle activation and loss in human ovaries post chemotherapy

D. Shai[1], H. Roness[1], I. Spector[1], H. Raanani[1], S. Aviel-Ronen[2], M. Shapira[1], M. Baum[1], I. Gat[1], D. Meirow[1]

[1]Sheba Medical Center, Morris Kahn Fertility Preservation Center, TEL AVIV, Israel ;
[2]Sheba Medical Center, Department of Pathology and Talpiot Medical Leadership Program, Tel aviv, Israel

Study question: What are the immediate pathophysiological findings related to follicle survival, dynamics and stromal injury in ovaries of patients treated with alkylating and non-alkylating agent chemotherapy.
Summary answer: Follicle activation and not primordial follicle apoptosis or stromal fibrosis may be an underlying mechanism of chemotherapy-induced follicle loss in patients treated with alkylating agents.
What is known already: Alkylating agents are the most ovotoxic of the chemotherapy agents, causing significant loss of ovarian follicle reserve, however very little data exists to explain the mechanisms underlying this loss in the ovaries of patients exposed to chemotherapy treatments Some mechanisms have been suggested, such as apoptosis of primordial follicles, stromal and blood supply changes and accelerated activation of primordial follicles. Examining the acute changes in human ovaries recently exposed *in vivo* to alkylating and non-alkylating chemotherapy will increase our understanding of the mechanisms of chemotherapy--induced follicle loss and enable development of targeted Fertility Preservation strategies.
Study design, size, duration: Histological analysis of ovarian cortical tissue removed from 96 women aged 15-39 with cancer diagnoses who were undergoing ovarian tissue cryopreservation for fertility preservation. Forty-eight had received chemotherapy treatment within six months of tissue retrieval; 24 with alkylating agents and 24 with non-alkylating agent chemotherapy. Ovarian cortical tissue from 48 age-matched patients who had not received chemotherapy were used as controls.
Participants/materials, setting, methods: Fresh ovarian tissue samples were paraffin embedded, serially sectioned and histologically evaluated for follicle numbers by three independent researchers. Immunohistochemical staining was conducted for apoptosis (cleaved Caspase-3), FOXO3a, collagen (Sirius red) and neovascularization (CD34).
Main results and the role of chance: Ovaries of women treated with alkylating chemotherapy contained significantly more growing follicles compared with age-matched controls (mean of 4.2 ± 0.6 growing follicles per section compared to 2.2 ± 0.3, $p=0.004$). The number of growing follicles was significantly inversely correlated with time from treatment ($r = -0.52$, $p=0.009$), such that the highest number of growing follicles was seen in ovaries removed within a few days of chemotherapy exposure. Despite a significant drop in primordial follicle population in ovaries treated with alkylating chemotherapy, no increased expression of apoptosis marker cleaved Caspase-3 was seen in primordial follicles even within a week post chemotherapy, while increased staining of cleaved Caspase-3 was observed in granulosa cells of growing follicles. Decreased nuclear staining of FOXO3A was seen in oocytes of PMFs in ovaries treated with alkylating agents compared with untreated ovaries. Marked fibrosis and

neovascularization was observed in ovaries exposed to alkylating agent chemotherapy 4-6 months earlier, but was not seen as acute changes in ovaries exposed more recently.
Limitations, reasons for caution: We were unable to perform molecular analysis on human ovarian samples as we had access to paraffin embedded not fresh-frozen samples. Therefore, the study was restricted to histological evaluations.
Wider implications of the findings: This study provides evidence that chemotherapy induces follicle activation and loss *in vivo* in ovaries of patients treated with alkylating agents. Prevention of this increased follicle activation may therefore be a potentially successful and highly targeted method of fertility preservation, which would not interfere with the effectiveness of chemotherapy.
Trial registration number: Helsinki #8065-10-SMC

P-409 Fertility preservation and oocyte quality in women with breast cancer and ovarian tumors: a prospective analytical study.

C. Fabiani[1], A. Guarino[1], E. Licata[1], G. Paciotti[1], C. Meneghini[1], R. Rago[1]

[1]ASL ROMA 2 - Sandro Pertini Hospital, REPRODUCTION PHYSIOPATOLOGY and ANDROLOGY, roma, Italy

Study question: Does the type of cancer disease influence the oocyte quality and ovarian response to stimulation for fertility preservation (FP) in female oncology patients?
Summary answer: Fewer mature oocytes are retrieved in ovarian and breast cancer patients than infertile patient, the number of dysmorphic oocytes is higher with a recurrent abnormality.
What is known already: Cancer survival has improved significantly and maintaining fertility is an important factor for the quality of life and for the management of cancer patient. Breast cancer is the most frequent cancer in women, and ovarian cancer, specially border-line one, is increasing in young patients. Currently mature oocyte cryopreservation is a standard technique for fertility preservation. The negative impact of cancer therapy on fertility is well known. However the data on the pattern of ovarian response after ovarian stimulation in oncology patients are limited. Few studies analyzed the effect of the type of malignancy on oocyte quality, in cancer patients.
Study design, size, duration: This is a prospective analytical study performed in the IVF unit at the Sandro Pertini Hospital in Rome between 2016 and 2019. The aim of this study is to investigate the effect of cancer diseases on number, above all on quality of oocyte and on dysmorphic oocyte ratio, in women with breast cancer and ovarian tumors compared to women age-matched who undergone ICSI treatment for tubal or male factor infertility.
Participants/materials, setting, methods: A total of 64 patients recently diagnosed with breast cancer (Group A) and 14 patients (Group B) with ovarian tumors were referred for counseling on FP. A total of 168 patients under 38 age underwent ICSI treatment for tubal or male infertility is the control group (Group C). Baseline characteristics are age, body mass index (BMI) and antimulleran hormone (AMH) value. The primary outcome was number and quality of retrievd oocyte from ovarian pick-up (OPU).
Main results and the role of chance: Baseline characteristic are comparable. Despite similar ovarian stimulation protocol, similar mean stimulation duration (Group A: 10.72; Group B: 10.4; Group C: 11.05), the recombinant FSH cumulative dose was significantly higher in breast patients (Group A: 2103.61 U.I.; Group C: 1514.77 U.I.) and the mean oestradiol peak at triggering was significantly lower due to the administration of letrozole in this patients (Group A: 597.16; Group C: 1131.49). Also the number of MII was significantly lower in cancer patients (Group A: 68.7%; Group B: 72.1%; Group C: 78.8%), the number of immature oocytes dose was significantly higher in breast cancer (Group A: 21.7 %; Group C: 13.5%), while the ratio of dysmorphic oocyte was significantly higher in cancer patients, expecially in ovarian cancer ones (Group A: 9.6% ;

Group B 16.5%; Group C: 7.7%). In Group A and B dysmorphic analyzed oocyte have a particular abnormality: a perivitelline space (SPV) with granularity.
Limitations, reasons for caution: Limitations concern the paucity of specific cancer group, especially ovarian tumors and BRCA mutated breast cancer patients. We still have not a follow up data to evaluate the competence of vitrified MII oocytes for oncology patients and we cannot report information on spontaneous conceptions and births.
Wider implications of the findings: Fertility outcomes in oncology patients have not been adequately studied due to the small number of patients with cancer undergoing FP. Few studies investigated the pattern of follicular growth to better characterize the outcome of ovarian stimulation not only in oocyte number and maturity, but also in oocyte quality.
Trial registration number: not applicable

P-410 Infertility and access to parenthood after an adolescent and young adult (AYA) cancer: a second 'obstacle course'?

M. Vialle[1], J. Saïas-Magnan[2], P. Bottin[2], A. Dezamis[3], C. Metzler-Guillemain[2], A.D. Bouhnik[4], J. Mancini[4], B. Courbiere[2,5]

[1]Centre Norbert Elias UMR 8562, Ecole des Hautes Etudes en Sciences Sociales EHESS- Centre National de la Recherche Scientifique CNRS, Marseille, France ; [2]Hôpital de la Conception - AP-HM, Department of Reproductive Medicine / CECOS, Marseille, France ; [3]Institut Paoli Calmettes, Department of Clinical Psychology, Marseille, France ; [4]Sciences Economiques et Sociales de la Santé & Traitement de l'Information Médicale SESSTIM, Institut National de la Santé et de la Recherche Médicale INSERM U125- Aix-Marseille Université- Institut de Recherche pour le Développement IRD UMR 259, Marse ; [5]Aix Marseille Univ, Avignon Univ- CNRS- IRD- IMBE, Marseille, France

Study question: What are the experiences of the medical care of fertility and access to parenthood for French adults in age to procreate who have had an AYA cancer?
Summary answer: Become parent after an AYA cancer is still a difficult pathway: patients report information and medical support gaps, feel guilty or are sometimes blamed.
What is known already: The medical literature has so far mainly shown the important effects of cancer treatments on fertility, the positive results of fertility preservation methods, or the uncertain conditions for maintaining or regaining fertility after cancer. Research in the humanities and social sciences has more specifically analysed the difficulties experienced when cancer is diagnosed and during treatment. Few have studied the post-cancer experiences of access to parenthood according to fertility conditions. The few existing surveys are quantitative, but none are based on in-depth qualitative interviews.
Study design, size, duration: This study was conducted between December 2018 and November 2019 and was carried out by a multidisciplinary team of sociologists and doctors. A qualitative survey was performed through in-depth interviews with 22 women and 14 men, who were adolescents or young adults at the time of cancer diagnosis and in remission for at least one year. Three types of cancer were studied: breast, testicles and malignant hematological diseases.
Participants/materials, setting, methods: Respondents were recruited from different locations and by different means: oncology or reproductive departments, snowball sampling, testimony call. Interviews were conducted by a sociologist face to face, by visioconference or telephone. The interviews covered, in a retrospective and chronological way: the personal, conjugal and professional history, the occurrence of cancer and its impact, especially in relation to treatment. The effects on fertility, desire for children and access to parenthood were discussed throughout the narrative.
Main results and the role of chance: A total of 36 interviews ranging in length from one to three hours were conducted with 22 women and 14 men, aged between 22 and 45 years. Among them, 8 women had breast cancer and 14 had malignant hematological disease; 6 men had testicular cancer and 8 had malignant hematological disease. Among women, 9 underwent a fertility preservation before cancer treatment, 2 after and 9 did not have preservation. Among the men, 13 did sperm cryopreservation and 1 did not. Among the 22 women interviewed, 8 had a child since the end of their cancer treatment, conceived naturally or by assisted reproductive technologies (ART) with their cryopreserved or donated oocytes; 6 of 14 men had or were expecting a child, conceived naturally or by ART with their cryopreserved or donated sperm. A few knew if they were fertile or infertile at the time of interview, but most were unaware of their fertility condition. This diversity of situations makes it possible to analyse a variety of experiences of medical care after cancer. However, despite this diversity, difficulties linked information or medical support gasps are systematically reported, appearing at different stages of the pathway towards parenthood according to the respondents.

Limitations, reasons for caution: This qualitative study is based on a limited number of interviews and a sample of people with limited characteristics. A study with a larger sample of people with broader characteristics would allow to reinforce or discuss the results. A selection bias is present, related to the volunteering process to participate.
Wider implications of the findings: The results showed the importance of a broader diffusion to the medical community and patients of the post-cancer existing 'self-reconstruction' difficulties, related to the treatment consequences on fertility, sexuality and couple life and the lack of information and medical support towards parenthood.
Trial registration number: Not applicable

P-411 Hydrogel derived from decellularized bovine ovarian extracellular matrix supports human follicle survival in vitro

M.C. Chiti1, L. White[2], J. Vanacker[1], E. Ouni[1], A. Des Rieux[3], M.M. Dolmans[1,4], C. Amorim[1]

[1]Institut de Recherche Expérimentale et Clinique, Pôle de Recherche en Gynécologie- Universitè Catholique de Louvain, Brussels, Belgium ;
[2]University of Nottingham, Wolfon Centre for Stem cells- Tissue Engineering and Modeling, Nottingham, United Kingdom ;
[3]Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials Unit-Université Catholique de Louvain, Brussel, Belgium ;
[4]Cliniques Universitaries St. Luc, Départemnt de Gynecologie, Brussel, Belgium

Study question: Is a thermosensitive hydrogel derived from decellularized bovine ovarian extracellular matrix (boECM) able to support human follicle survival and development after 7 days of in vitro culture?
Summary answer: When combined with alginate, this thermosensitive hydrogel constitutes what appears to be an efficient three-dimensional (3D) matrix for in vitro culture of human ovarian follicles.
What is known already: To successfully assemble a working artificial ovary, we need to create a 3D matrix able to accommodate isolated follicles and cells. While encouraging results have been achieved with fibrin matrices, the next step is designing an ECM-derived scaffold more closely resembling human ovarian ECM in its biochemical composition. This new generation of biomimetic scaffolds might well prove to be a breakthrough for ovarian follicle survival in an artificial ovary prototype.
Study design, size, duration: Two hydrogels (boECM1, boECM2) were analyzed for dsDNA, collagen and glycosaminoglycans (GAGs), and compared to bovine ovarian tissue. Human ovarian tissue (n=3 patients) was then digested to isolate ovarian follicles and encapsulate them inside 4 selected hydrogel combinations: 1) 100% boECM2; (2) 90% boECM2 + 10% alginate; (3) 75% boECM2 + 25% alginate; (4) 100% alginate (control). Follicle count, viability and growth were evaluated on day 0 (D0) and D7 after in vitro culture.
Participants/materials, setting, methods: Ovarian biopsies were obtained from fertile patients (n=3) with no ovarian pathology, and live/dead assays were performed to evaluate follicle survival before in vitro culture. Follicle recovery rates (n follicles encapsulated on D0/n follicles recovered on D7) were assessed after one week. Follicle growth was measured by comparing mean follicle diameters between D0 and D7 of in vitro culture. On D7, follicle viability was determined using a live/dead assay kit.
Main results and the role of chance: While dsDNA was significantly reduced in both boECM1 and boECM2 ($p<0.0001$) compared to native tissue, the greatest reduction ($p<0.01$) was observed in boECM2. There was no difference in collagen content between groups. GAG content was significantly higher ($p<0.01$) in native tissue than in boECM1, but not boECM2. Indeed, a significantly higher concentration of GAGs was found in boECM2 (P-value <0.05). A total of 114 isolated human follicles were encapsulated in boECM2-derived hydrogels and in vitro cultured for 7 days. On D7, overall follicle recovery rates were: 0%, 23%, 65% and 85% in groups 1 to 4 respectively, rising proportionately with increased alginate content. There was no difference in follicle viability between group 2 and 3 and the control group (group 4) (87%, 97% and 87% respectively) but follicle viability was not applicable in group 1 as no follicles were recovered. On D7, statistically significant follicle growth was accordingly detected in all groups except group 1 (P-value <0.05, P-value <0.01, P-value <0.001 respectively). Moreover, similar growth rates were found compared to the control group on D7.
Limitations, reasons for caution: This was a pilot study.
Wider implications of the findings: boECM2 combined with alginate may prove to be a promising new scaffold material for creation of an artificial ovary for women in remission from cancer, but at risk of malignant spread from reimplantation of their tissue.
Trial registration number: not applicable

P-412 In depth follow up of childhood development in two generations of spermatogonial stem cell transplantation derived offspring in a mouse model

J. Serrano[1], R. Van Eekelen[1], C.M. De Winter-Korver[1], S.K.M. Van Daalen[1], C.L. Mulder[1], A.M.M. Van Pelt[1]

[1]Amsterdam UMC- University of Amsterdam, Centre for Reproductive Medicine, Amsterdam, The Netherlands

Study question: What is the effect of transplantation of *in vitro* propagated spermatogonial stem cell (SSCT) on neonatal health and development in the derived offspring?
Summary answer: Systematic preclinical safety testing of SSCT derived offspring reveals no major differences in congenital abnormalities, birthweight, physical development and reflex ontogeny of the derived offspring.
What is known already: Pediatric cancer treatments improved greatly in recent years leading to increased life expectancy, but infertility is a late effect. Spermatogonial stem cells (SSCs) enable long-term male fertility through a tight balance between self-renewal and differentiation in the testis. To preserve fertility in prepubertal male cancer patients, a testicular biopsy is offered prior to gonadotoxic treatment. SSC *in vitro* propagation followed by auto-transplantation is proposed to restore spermatogenesis allowing natural conception. As several assisted reproductive techniques are linked to increased congenital abnormalities, low birthweight or developmental deviations, detailed pre-clinical follow-up of development of SSCT offspring is warranted before clinical application.
Study design, size, duration: Mouse neonatal SSCs were propagated *in vitro* and subsequently transplanted into sterile males (n=30). Control and fertile transplanted males (n=3 per group) were bred with control females (n=27) for one (control) and two generations (SSCT) of offspring, powered for congenital abnormalities (n=144 control, n=124 F1 SSCT, n=117 F2 SSCT). To assess childhood development, the pups were checked (double blinded) for congenital abnormalities, physical and behavioral development during the first 28 days of life.
Participants/materials, setting, methods: SSCs were isolated from neonatal DBA/2J mice and propagated *in vitro*. Sterile males (W/W-v) received SSCT to recover fertility. Control (DBA/2J) and transplanted (W/W-v) males were placed in breeding with control females (DBA/2J). At birth, the pups were checked for congenital abnormalities, birthweight and length. During the first 28 days, birth defects, ear opening, eye opening, complete fur growth, incisor eruption were checked, along with behavioral reflex testing of negative geotaxis, grasp and righting reflexes.
Main results and the role of chance: Overall, the health and development of naturally conceived SSCT derived pups during the first 28 days of life was similar to control pups, both in the first and second generations. However, statistically significant physical differences were found in the birthweight of second-generation pups, with a 0.068g decrease from 1.18 grams in control to 1.11 grams in SSCT derived offspring (95%CI: [-0.134; -0.003]). Furthermore, the eruption of the upper incisor occurred 1.16 days earlier in SSCT derived pups of the second generation (95%CI: [-2.161; -0.167]). No statistically significant differences were found between control and SSCT derived offspring, in both generations, for the remaining physical and behavioral assessments: birth length, eye and ear opening, lower incisor eruption, fur growth, righting reflex, grasping reflex and negative geotaxis. The study was powered for congenital abnormalities, which was a rare event (n=8), with no statistically significant differences found between groups in either generation (control n=1/142, SSCT F1 n=3/121, SSCT F2 n=4/113). However, the estimated odds ratios were relatively high at 3.57 (95%CI: [0.13; 293.33]) for F1 and 4.19 (95%CI: [0.60-82.95]) for F2.
Limitations, reasons for caution: Although powered for congenital abnormalities, the sample size of this study was insufficient to determine the impact of SSCT in the incidence in the offspring, given the rarity of congenital abnormalities.

Wider implications of the findings: Given the overall normal development of SSCT derived offspring, and after improvement of *in vitro* propagation of human SSCs and development as an advanced therapeutic medical product (ATMP), ethical approval should be requested for introduction of SSCT in a phase 1 clinical trial, alongside follow up of the children.
Trial registration number: Not applicable.

P-413 Modified RNA Encoding for Anti-Müllerian Hormone Has Fertoprotective Potential in Human Ovary Exposed to Cyclophosphamide

L. Man, M.D.- M.Sc.[1], E. Kallinos[1], Z. Rosenwaks[1], D. James[1]

[1]Weill Cornell Medical College, Center for Reproductive Medicine, New York City, U.S.A.

Study question: Does the administration of modified RNA encoding AMH confer a fertoprotective effect on the primordial follicle pool in the context of cyclophosphamide?
Summary answer: Administration of modified RNA AMH conferred a fertoprotective effect from cyclophosphamide, preserving the primordial follicle pool in both mouse ovary and human ovarian cortical xenografts.
What is known already: The risk of ovarian failure post-chemotherapy is determined largely by the type, amount of chemotherapy, and the age of the patient at treatment (Meirow, et al., 2010). Several studies have investigated the fertoprotective potential of a wide range of compounds in rodents, non-human primates, and patients (Spears, et al., 2019). In mice, recombinant or adenovirus-encoded AMH has been shown to protect the ovary from cyclophosphamide (Cp)-induced follicle loss (Kano, et al., 2017, Sonigo, et al., 2019).
Study design, size, duration: Cross-sectional study.

Murine model: intraovarian injection (saline/murine Mod-RNA-AMH) followed by an intraperitoneal (IP) injection 24 hours later (saline/Cp). A total of 4 arms, 6 ovaries in each.

Xenotransplantation model: human ovarian tissue transplanted into immunocompromised mice, intra-graft injection (buffer/human recombinant-AMH/human Mod-RNA-AMH) followed by an IP injection 24 hours later (saline/Cp). A total of 4 arms, 4 grafts in each.

Ovaries/grafts were harvested 2 weeks after the first chemotherapy injection.
Participants/materials, setting, methods: In the murine model, we used 6-7-week-old C57/B6 females.

In the xenograft model, we co-transplanted human ovarian cortical tissue from a 12-year-old organ donor, with endothelial cells, into NOD *scid* gamma (NSG) mice.

Cp was administered at the dose of 60mg/Kg, and the protocol was repeated a week later.

After harvest of the ovaries/xenografts, the ratio of follicles of all growth stages was measured in histologic sections.
Main results and the role of chance: In the murine model, we found the retention of primordial follicles similar to the controls: saline-intraovarian/saline-IP, 49±12.10, Mod-RNA-AMH/saline, 53.33±7.81, compared to Mod-RNA-AMH/Cp, 47.17±13.61. A 50% decrease was noted with saline/Cp Tx: 26.5±9.01. In the human xenografts, the retention of early primordial follicles was markedly improved with Mod-RNA-AMH pre-treatment. Buffer intra-graft/Cp IP 9.41±10.91% primordial follicles versus 37.48±17.12% with Recombinant-AMH, and 56.24±39.44% with Mod-RNA-AMH. Notably, the percentage of primordial follicles was similar to grafts treated with buffer/saline: 51.67± 4.04%.
Limitations, reasons for caution: Human ovarian tissue available for research is restricted. Repeating the experiment with more replicates will strengthen the results. Moreover, demonstrating the benefit to human xenografts at later time points will provide further support for the positive effect conferred by AMH.
Wider implications of the findings: Due to its high efficiency, transient protein expression, and the fact that it does not elicit a substantial innate immune response, Mod-RNA is an optimal mode of delivery. Administration before chemotherapy may confer a pronounced fertoprotective effect with minimal expense and intervention.
Trial registration number: na

P-414 impact of cancer treatment on ART outcomes: study of male cancer survivors

S. Shibasaki[1,2], Y. Takeshige[1,2,3,4], H. Hattori[1,2], Y. Nakamura[1,2], N. Aono[1,2,3,4], Y. Nakajo[1], N. Okuyama[2,3], T. Tai[1,3], M. Toya[1], T. Hashimoto[2,3], H. Igarashi[1], K. Kyono[1,2,3,4]

[1]Kyono ART Clinic Sendai, Department of Gynecology, Sendai, Japan ; [2]Human Ovariantissue Preservation Enterprise, Department of Gynecology, Tokyo, Japan ; [3]Kyono ART Clinic Takanawa, Department of Gynecology, Tokyo, Japan ; [4]Kyono ART Clinic Morioka, Department of Gynecology, Morioka, Japan

Study question: Does chemotherapy and/or radiation therapy in male cancer patients affect the fertility potential of sperm, embryonic development, or clinical outcomes?
Summary answer: There was no significant difference in embryonic development or clinical outcomes between the pre-cancer treatment group and the post-cancer treatment group.
What is known already: It is well known that cancer treatment, particularly chemotherapy and radiation therapy, for male cancer patients have negatively affected spermatogenesis. For this reason, sperm cryopreservation is recommended before cancer treatment to preserve fertility. However, it is not clear whether chemotherapy and radiation therapy affect embryonic development and clinical outcomes of ART.
Study design, size, duration: This study included the patients who used ART from January 1997 to June 2019. We defined 25 patients who underwent 45 ICSI cycles with cryopreserved ejaculated sperm before cancer treatment as group A. Of 109 patients who visited our clinic after cancer treatment, 31 cancer survivors who underwent 70 ICSI cycles with ejaculated sperm were defined as group B. A total of 63 frozen embryo transfers were performed (group A: 27, group B: 36 cycles).
Participants/materials, setting, methods: Of the cancer survivors, sperm could not be collected in 42 patients and ICSI was performed in 31 patients. Only semen analysis was performed for the rest. The cancer treatment was performed by chemotherapy and/or radiation therapy. Semen parameter, embryonic development, clinical outcomes were compared between groups A and B. Chi-squared test, Mann-Whitney U test, and Fisher's exact test were used for statistical analysis. $P<0.05$ was considered statistically significant.
Main results and the role of chance: The major cancer types in group A and B were testicular tumor (40.0%, 25.8%) and hematopoietic malignancy (28.0%, 51.6%). The mean age of females at the ovum pick-up in group B (37.1±4.5) was significantly higher than in group A (35.3±4.0) ($P<0.05$). There were no significant differences in the anti-Mullerian hormone levels or antral follicle counts, or baseline FSH levels. The mean age of males at the sperm collection in group B (39.9±7.9) was significantly higher than in group A (35.9±6.4) ($P<0.05$). In group B, the mean period from the end of cancer treatment to the start of ART was 10.8±9.7 years. The median of sperm concentration in group A [37.7×10^6 (20.5-78.9)] was significantly higher than in group B [5.5×10^6 (0.9-26.4)] ($P<0.05$). Two cases in group A showed azoospermia after cancer treatment. There were no significant differences in fertilization rates [63.5% (167/263), 65.7% (229/326)], and blastocyst formation rates [48.7% (73/150), 49.3% (75/152)] between groups A and B. Similarly, there were no significant differences in the pregnancy rates of frozen-thawed embryo transfer [59.3% (16/27), 50.0% (18/36)] and live-birth rates [44.4% (12/27), 41.7% (15/36)] between groups A and B.
Limitations, reasons for caution: In this study, the ART outcome was compared between the patients with sperm frozen before cancer treatment, and the only patients with sperm obtained after the treatment, which might cause a certain bias in grouping. We could not have enough information about cancer treatment, which should be shared by oncologist.
Wider implications of the findings: Considering the effects of gonad toxicity from chemotherapy treatment, sperm cryopreservation before cancer treatment is basically recommended. However, if sperm can be collected after a withdrawal period, ART treatment outcomes might be comparable. Since the number of target patients was still small, further study would be necessary.
Trial registration number: not applicable

P-415 No sign of actin polymerization or Hippo pathway inhibition in fragmented human ovarian tissue

S. Aagaard Lunding[1], S.G. Kristensen[2], L. Hardardottir[2], H.Ø. Olesen[2], A. Nyboe Andersen[1], C.Y. Andersen[2], S.E. Pors[2]

[1]Rigshospitalet, The Fertility Clinic, Copenhagen, Denmark ;
[2]Rigshospitalet, Laboratory of Reproductive Biology, Copenhagen, Denmark

Study question: Does tissue fragmentation of human ovarian cortex result in polymerization of the actin cytoskeleton and subsequent activation of follicle growth through Hippo pathway inhibition?
Summary answer: Tissue fragmentation of human ovarian cortex did not result in actin polymerization nor did it result in follicle activation by inhibition of the Hippo pathway.
What is known already: The Hippo pathway has been associated with regulation of early ovarian follicle growth in mammals including humans. Studies of murine ovaries suggest that changes in the actin cytoskeleton, caused by fragmentation, result in inhibition of the Hippo pathway by dephosphorylation of YAP and in turn may activate follicle growth by transcription of growth factors (eg. CCN's) and apoptosis inhibitors. In humans, *in vitro* and *in vivo* studies of fragmented ovarian tissue demonstrated upregulation of growth factors and growth of preantral follicles. However, the connections between fragmentation, the actin cytoskeleton and follicle activation in humans are yet to be confirmed.
Study design, size, duration: Donated frozen ovarian cortex from six women (34-37 years), undergoing ovarian tissue cryopreservation for fertility preservation between 2001 and 2011 prior to gonadotoxic therapy, were thawed for *in vitro* and xenotransplantation study. From each woman one cortex piece of 5x4x1mm was fragmented into 20 cubes of 1x1x1mm and another intact piece served as control tissue. Both fragmented and control tissue were incubated for either 0, 10, 30, 60, 120 or 240 minutes prior to examination.
Participants/materials, setting, methods: Actin polymerization was assessed with western blot of the ratio of F-actin to G-actin in fragmented and control ovarian tissue. Inhibition of the Hippo pathway was assessed with western blot of the ratio of phosphorylated YAP (pYAP/YAP) and gene expression analysis of the downstream growth factors CCN2, CCN3 and CCN5 in fragmented and control ovarian tissue. Fragmented and control tissue were xenografted to immunodeficient mice for six weeks before histological assessment of follicle growth.
Main results and the role of chance: Both F-actin and G-actin were detectable in all samples, but no significant difference was found in the ratio in fragmented tissue compared with control tissue for each timepoint (p=0.8). Both YAP and phosphorylated YAP (pYAP) were expressed in all samples. At the three selected timepoints (0, 60 and 120 minutes) there were no difference in the ratio of YAP/pYAP in fragmented and control tissue (p=0.6). Moreover, CCNs were expressed in all tissue samples. Only CCN5 was on average 0.56-fold lower in control tissue compared with fragmented tissue (p=0.0026). However, when assessing CCN5 in the fragmented tissue at the different timepoints, expression of CCN5 did neither increase nor decrease over time (p=0.8). Thus, fragmentation did not induce upregulation of CCN5 gene expression. Histological assessment of the xenografted ovarian tissue revealed fewer follicles in the fragmented tissue compared with control tissue, 239 versus 975 preantral follicles, respectively. A logistic regression model accounting for paired samples showed that the proportions of growing follicles, defined as all non-primordial follicles, in the exposed tissue (34.7%) and the control tissue (27.4%) were not different (p = 0.56).
Limitations, reasons for caution: Donated ovarian tissue were only available from six women, which limits the statistical power. Initially, ovarian cortex is cut into pieces prior to cryopreservation and some activation could have been induced in the control tissue. Furthermore, the unequal follicle distribution throughout the cortex warrants caution when interpreting the findings.
Wider implications of the findings: We were not able to confirm that fragmentation of human cortical tissue results in actin polymerization and subsequent follicle activation through disturbance of the Hippo pathway. Thus, these *in vitro* and *in vivo* results may indicate that ovarian tissue fragmentation is an ineffective method to activate follicle growth in humans.
Trial registration number: Not applicable

P-416 Ovarian stimulation for fertility preservation in young women: is there a right time to start?

R. Quintana Berto[1], A. Santolaria Baig[1], N. Garcia Camuñas[1], J. Subirá Nadal[1,2], P. Polo Sanchez[1], J.M. Rubio Rubio[1]

[1]La Fe University Hospital, Women's Health Area, Valencia, Spain ;
[2]IVI-RMA Castellon-Valencia, Reproductive Endocrinology, Castellon, Spain

Study question: Is there an optimal time to start an ovarian stimulation in young women who initiate an oocyte cryopreservation (OC) cycle for fertility preservation (FP)?
Summary answer: Starting OC cycles out of the early follicular phase (EFP) – or random-start protocol – may provide better ovarian response and oocyte yield.
What is known already: OC is the preferred method for FP compared to other treatments. Traditionally, stimulation protocols start during the EFP but this methodology sometimes requires more time depending on patient's ovarian cycle. However, in certain settings – cancer, for instance – we do not have enough time for delaying other therapies in order to preserve women's fertility. Some authors have argued that is possible to achieve a synchronized follicular development and an optimal oocyte retrieval despite starting the stimulation out the EFP based on the different follicles waves. Random-start ovarian stimulation protocols are a feasible alternative to conventional cycles in such scenery.
Study design, size, duration: This is a retrospective study performed from January 2013 to January 2020 at a tertiary university hospital. Our research was focused in the first OC cycle of young women (< 25 years old) which are expected to have a better cycle response due to their clinical characteristics. Data was retrieved from patients' electronic medical record.
Participants/materials, setting, methods: We collected data from epidemiology, stimulation and oocyte retrieval from a total number of 78 OC cycles. Two groups were stablished depending on the phase in which the treatment began: EFP n=63 and random-start n=15. Continuous variables were summarized by the median and standard deviation for a descriptive analysis. After evaluating the normal distribution of both groups, we performed a nonparametric statistical analysis to compare them.
Main results and the role of chance: Both groups were comparable in terms of Body Mass Index (BMI), Anti-Müllerian Hormone (AMH) or Antral Follicular Count (AFC).

Total dosing of FSH was similar in both groups with a mean of 2401,441 ± 1044,6 IU in the EFP group and 2295 +/- 636,90 IU in the random-start grouP- (p=0.941).

The total number of follicles at triggering day was comparable in both groups. However, there was a higher number of follicles >16 mm in the random-start group (12 ± 5) compared with the EFP group (7,47 ± 5) (p=0,011). Estradiol values at the trigger day were similar, as well as Follicular to Oocyte Index (FOI) and Follicular Output Rate index (FORT).

Nevertheless, the oocyte retrieval was more successful in the random-start group with a mean number of 11,72 (±7,25) oocytes in the EFP patients and 17,43 (±9,84) in the random-start patients (p=0.018). Even though the maturity rate was comparable in both groups, the number of metaphase II oocytes was also higher in the random-start group (p= 0,017).
Limitations, reasons for caution: The data from this study was retrospective and from a single center. Further studies including more patients would ensure a stronger statistically significant difference.
Wider implications of the findings: Random-start protocol for OC cycles provides an advantage in terms of timing. In settings in which an urgent ovarian stimulation is needed, starting it without delays not only allows to attempt fertility preservation for patients who have important time constraints, but also, does not compromise oocyte retrieval.
Trial registration number: Not applicable

P-417 Effect of BRCA genetic status on ovarian stimulation response for fertility preservation in breast cancer patients. A retrospective cohort study

L. Perez Martin[1], C. Alonso Mayo[1], S. Caballero Sanz[1], F.J. Amor Valera[1], D. Trobo Marina[1], F.M. Pérez Milán[1]

[1]Hospital General Universitario Gregorio Marañón, Reproduction Unit, Madrid, Spain

Study question: Do BRCA1 and BRCA2 mutation carriers diagnosed with breast cancer have an increased risk of poor ovarian response to ovarian stimulation for fertility preservation?
Summary answer: No association was observed between the presence of a BRCA gene mutation and ovarian response to ovarian stimulation in terms of number of oocytes retrieved.
What is known already: Breast cancer is the second most common malignancy diagnosed worldwide. 5-year survival rate extents to more than 80% due to early detection with screening programmes and the recent advances in its treatment. Successful treatment often requires the use of gonadotoxic chemotherapeutic agents, so fertility preservation is now advised to most premenopausal patients. Predisposing mutations are present in around 10-15% of cases, being BRCA genes the most commonly involved. There have been reports suggesting a poorer response to ovarian stimulation of BRCA mutation carriers, and recent studies showed lower serum AMH levels compared to non-carriers.
Study design, size, duration: This is a retrospective cohort study. All breast cancer patients who underwent fertility preservation in our unit from January 2010 to December 2019 were eligible to be included. Our aim was to compare the number of oocytes retrieved between BRCA mutation carriers and tested-negative controls. Secondary outcomes were number of metaphase II oocytes retrieved, rate of low or suboptimal ovarian response (<6 and < 9 oocytes, respectively) and cancelation rate due to insufficient response.
Participants/materials, setting, methods: All breast cancer patients tested for BRCA mutations who underwent a fertility preservation treatment were included in the study. We compared the number of oocytes retrieved in exposed and non-exposed patients using appropriate statistical tests. Effect size was estimated by relative risk, which was adjusted considering potential confounders by multivariate analysis (logistic regression). The study was conducted in Gregorio Marañón General University Hospital (Madrid, Spain), and was approved by the internal institutional committee.
Main results and the role of chance: We included 113 patients. BRCA mutations were present in 29 (25.7%). No differences were observed between groups regarding age, smoking habit, previous infertility and/or pregnancy, antral follicle count (AFC), initial and total dose of FSH consumed during ovarian stimulation and cotreatment with LH and/or letrozole.

No association was detected between BRCA status and number of oocytes retrieved (mean difference: 0.65; 95% CI: -2.29—3.59; p>0.05; log rank test p value >0.05). Multivariate analysis did not show association after adjusting by age, AFC, smoking habit and total FSH dose (regression coefficient: -0.99; CI 95%: -3.62—1.64).

BRCA status showed no effect on number of metaphase II oocytes retrieved (mean difference: 1.71; 95% CI -0.70—4.12; log rank test p value >0.05).

Cancelation rate due to insufficient response was similar in both groups (6.1% vs 9.3%; p >0.05). Risk of low and suboptimal response was also similar (RR: 0.70 CI 95%: 0.28—1.74; and RR: 0.96 CI 95%: 0.57—1.60; respectively). The impact of BRCA genetic status on low and suboptimal response was not affected by cofactors such as age, smoking habit, AFC and total FSH dose (adjusted OR: 0.99 CI: 0.87—1.83; and OR: 0.39 CI 95%: 0.08—1.83, respectively).
Limitations, reasons for caution: BRCA status was unavailable in many cases. The low number of patients included rendered a low power to conclude about the main objective, despite observed frequency of BRCA mutations being higher than expected. Pre-treatment AMH levels were unknown in most patients, so ovarian reserve could only be assessed by AFC.
Wider implications of the findings: As more BRCA mutation carriers are aware of their genetic status, more couples are seeking preimplantation genetic testing, which ideally requires a large number of oocytes and embryos. According to our findings, BRCA mutation carriers do not require oocyte cryopreservation to maximize chances before they decide to have children.
Trial registration number: Not applicable

P-418 Ovarian reserve before and after chemotherapy in triple negative breast cancer patients

J. Han[1,2], K. Hye In[1], K. Sung Woo[1,2], K. Hoon[1,2], K. Seung-Yup[1,2], S. Chang Suk[1,2], K. Seok Hyun[1,2], M.C. Young[1,2]

[1]Department of Obstetrics and Gynecology, Seoul National University Hospital, ; [2]Department of Obstetrics and Gynecology, Seoul National University College of Medicine,

Study question: Is there any difference in ovarian reserve after chemotherapy between triple negative breast cancer (TNBC) patients and other breast cancer patients?
Summary answer: Decreased ovarian reserve after chemotherapy is more obvious in TNBC group than other group. Early referral before chemotherapy is crucial especially in TNBC patients.
What is known already: Triple Negative Breast Cancer (TNBC) is a cancer that is estrogen receptor (ER) negative, progesterone receptor (PR) negative and human epidermal growth factor receptor 2 (HER2) negative, based on immunohistochemistry (IHC). Some studies suggest that women with BRCA1 or BRCA2 mutation have fewer oocytes in their ovaries and large portion of TNBC patients were proven to have BRCA mutation. But there is no report about association between TNBC and ovarian reserve.
Study design, size, duration: In this retrospective study, data from 138 breast cancer patients who referred for fertility preservation at a single tertiary center were examined from January, 2017 to December, 2018. All options were explained by specialist of endocrinology, and patients selected the method of fertility preservation. Most patients (n=122) chose GnRH agonist injections only, and other patients (n=16) wanted to perform oocyte or embryo cryopreservation.
Participants/materials, setting, methods: Subjects were classified into two groups, TNBC group (n=33) and other group (n=105) in which at least one of ER, PR, HER2 is positive. Clinical characteristics and markers of ovarian reserve (AMH, FSH, E2) of the two groups were compared before, 6 months, and 1 year after chemotherapy.
Main results and the role of chance: Baseline characteristics including age were not significantly different between two groups. In TNBC group, initial AMH levels were significantly lower than other group (3.25 ± 0.61 vs. 3.85 ± 1.04, p<0.001). After 1 year of chemotherapy, FSH levels were higher in the TNBC group (22.98 ± 13.08 vs. 11.15 ± 5.38, p=0.051).
Limitations, reasons for caution: Limitations of this study is that difference in drug regimen and duration of chemotherapy between two groups was not considered thoroughly. Further prospective studies with larger, homogenous cohorts are needed.
Wider implications of the findings: In this study, TNBC patients showed trend to have lower ovarian function before chemotherapy and slower recovery of ovarian function after chemotherapy. Therefore, urgent referral for fertility preservation should be considered especially in TNBC patients.
Trial registration number: not applicable

P-419 A streamlined andrology workflow for elective sperm freezing before chemotherapy is pertinent to timely preservation of male fertility

Z. Huang[1], J. Mathew[2], C. Ho[2], S. Chew[2]

[1]Institute of Molecular and Cell Biology- National University Health Systems, Obstetrics and Gynaecology, Singapore, Singapore Rep. of ; [2]National University Health Systems, Obstetrics and Gynaecology, Singapore, Singapore Rep. of

Study question: Does a streamlined andrology workflow help improve time-to-treatment for men who require elective sperm freezing before chemotherapy?
Summary answer: A standardized workflow with instructions and checklist for all clinicians to refer for elective sperm freezing before chemotherapy is efficient and reduce time to treatment.
What is known already: Men who require elective sperm freezing to preserve their fertility prior to chemotherapy to treat their medical conditions (e.g. cancer or debilitating medical conditions) are often referred to multiple clinics and specialists (referring physicians, fertility specialist and andrologist), undergo a multitude of investigations and go through many consent forms before receiving the appropriate treatment. This causes additional financial costs and further delays as well as precious time lost before these men can receive life-saving medical treatments. Many men become confused by conflicting instructions, frustrated by numerous appointments and lack accurate information about elective sperm freezing.

Study design, size, duration: Retrospective analyses on all the elective sperm freezing cases performed in 2018 was done to assess, 1. the duration required from time of referral to time of sperm freezing, 2. costs bore by these men and 3. types of cases referred. An institution-approved checklist and counselling form designed by the andrology service for elective sperm freezing is implemented from January 2019 and a prospective analysis of the referral process and outcomes was performed.

Participants/materials, setting, methods: Participants: Men referred from all disciplines for elective sperm freezing

Setting: The andrology service attends to men with a wide range of psychosexual and fertility issues; limited appointment slots available.

Method: An approved standardized checklist deployed in 2019 with indications, requirements and counselling form which a clinician (from any discipline) can access on the hospital internal web-portal to reference, download and employ in their clinics for direct onward referral to do elective sperm freezing for these men.

Main results and the role of chance: Elective sperm freezing requests comprises 20% the andrology service's workload. With the limited appointment slots and usually lengthy consults for psychosexual issues and complex andrological issues, it can result in long waiting times, increased number of clinic visits, increased costs and delays to life-saving treatment for these men just to access the service. Typically it takes up to 3 clinic visits before the men can schedule their elective sperm freezing prior to January 2019. Additionally, relevant investigations before sperm freezing such as sexually transmitted infections screen must be done as mandated by the Ministry of Health, Singapore - however prior to 2019, this was haphazard and not all men have their screen performed routinely - resulting in further delay to elective sperm freezing and initiating their medical treatment (range 4 -14 days). The referring physicians from multiple disciplines (medical-oncology, haematology, endocrinology, urology) had to delay commencing vital medical treatments for these men until their fertility preservation plans were completed. With the implementation of the streamlined workflow and checklist in January 2019, the referring physician can follow the checklist, counsel patients and refer directly for elective sperm freezing with no delay, waiting time or need to see the andrologist anymore.

Limitations, reasons for caution: The analysis is largely descriptive and applicable only in a tertiary referral hospital with a broad range of disciplines and high number of complex cases.

Wider implications of the findings: This resolves the unnecessary delay and extra costs for men to have elective sperm freezing done. The standardized checklist and counselling form will create a model of care for other clinical services to adopt. This is to avoid delay in commencing life-saving medical-treatment due to the need for fertility preservation.

Trial registration number: not applicable

P-420 Reproductive outcomes after IVF treatment in a cohort of Danish women transplanted with cryopreserved ovarian tissue

I.M. Dueholm Hjorth[1], S.G. Kristensen[2], M. Dueholm[3], P. Humaidan[4]

[1]The Regional Hospital in Horsens and Aarhus University Hospital, Department of Obstetrics and Gynecology, Aarhus C, Denmark ;
[2]The Juliane Marie Centre for Women- Children and Reproduction- University Hospital of Copenhagen- Blegdamsvej 9- 2100 Copenhagen- Denmark., Laboratory of Reproductive Biology, Copenhagen, Denmark ;
[3]Aarhus University Hospital, Department of Obstetrics and Gynecology, Aarhus, Denmark ;
[4]Skive Regional Hospital and Faculty of Health- Aarhus University, The Fertility Clinic and Department of Clinical Medicine, Skive and Aarhus, Denmark

Study question: How were the reproductive outcomes, ovarian stimulation regimens and predictive factors for successful fertility treatment in women undergoing ovarian tissue cryopreservation (OTC) and transplantation (OTT)?

Summary answer: Although low, reproductive outcomes after OTT are more favorable in younger patients swiftly referred to IVF treatment, followed by frozen-thaw embryo transfer.

What is known already: The reproductive outcomes after OTT are diverse as some women conceive spontaneously, whereas others fail to conceive even after repeated IVF treatment. Knowledge on the causes of this variability, including predictive factors for success is lacking, and limited data exists as to which ovarian stimulation and embryo transfer regimens are the most optimal for this group of patients.

Importantly, negative outcomes are generally underreported.

Study design, size, duration: Retrospective study in a cohort of 28 patients, undergoing OTT at Aarhus University hospital in Denmark followed by IVF treatment during the period 2012 to 2017.

Participants/materials, setting, methods: A cohort of 28 women undergoing IVF-treatment in Danish fertility clinics after OTC and OTT. The study included evaluation and analysis of patient characteristics, including diagnosis prior to OTC, details of the OTT surgery, ovarian stimulation regimens and reproductive outcomes, including number of follicles, oocytes, embryo transfers, pregnancy, and live birth. The possible impact of time to fertility treatment was also evaluated.

Main results and the role of chance: In 19 patients responding to ovarian stimulation, a median of 3.0 cycles per patient (range: 1-14 cycles) was performed and 2.0 mature oocytes were retrieved per cycle. Eleven women (39%) achieved 15 pregnancies of which 60% were lost during first or second trimester, resulting in 5 patients (17,9%) having one or more live births, and 7 healthy children being born. In breast cancer patients (mean age at OTC=33 years), the pregnancy (PR) and live birth rates (LBR) were 35.0% and 5.0% per embryo transfer, respectively. For all other diagnoses (mean age at OTC=26.6 years), PR and LBR were 50.0% and 37.5% per embryo transfer, respectively. None of 12 women aged ≥35 years at OTT achieved a live birth. A total of 1.42 fertilized oocytes per cycle, was obtained using the long GnRH agonist protocol compared to 0.71 fertilized oocyte per cycle in GnRH antagonist cycles (p=0.004, Mann-Whitney). Frozen-thaw embryo transfer resulted in more pregnancies than fresh embryo transfer (P<0.05). There were no significant differences in age at OTC, diagnosis or transplantation site between patients who had oocytes retrieved and non-responders to stimulation, but in non-responders, the mean time from OTT was 15.7 months compared to 9.9 months in responders (p<0.05).

Limitations, reasons for caution: Although this study includes the largest single center Scandinavian cohort of OTC-OTT patients undergoing IVF treatment, the sample is still low, limiting the statistical power. Moreover, since live birth was rare, parameters related to this outcome may be masked.

Wider implications of the findings: Based on the present results, the long GnRH agonist down-regulation protocol, followed by freeze-all and frozen-thaw embryo transfer should be recommended and multiple OTT procedures and repeated IVF-treatment may be necessary to achieve a live birth. Patients may benefit from being referred to fertility treatment soon after OTT.

Trial registration number: 3-3013-2790/1

P-421 Spermatogenesis enhancement in neonatal mouse frozen - thawed testis after 3- dimensional culture with growth factors.

A. Alrahel[1], M. Movahedin[2], Z. Mazaheri[3], F. Amid[4]

[1]Reproductive Biotechnology Research Center- Avicenna Research Institute- ACECR-Tehran- Iran., Reproductive Biotechnology Research Cente, tehran, Iran ;
[2]Anatomical Sciences Department- Faculty of Medical Sciences- Tarbiat Modares University- Tehran 14115331- Iran, Anatomical Sciences Department, Tehran, Iran ;
[3]Basic Medical Science Research Center- Histogenotech Company, Basic Medical Science Research Center- Histogenotech Company, Tehran, Iran ;
[4]Anatomical Sciences Department- Faculty of Medicine- Tehran University of Medical Sciences-, Anatomical Sciences Department, Tehran, Iran

Study question: Are growth factors (GF) provide profound impact on spermatogenesis after 8 weeks of in-vitro 3- dimensional culture?

Summary answer: The level of TnP1 gene expression and ACRBP were significantly higher in the fresh culture group with growth factors compared to control groups.

What is known already: Aggressive chemotherapy may lead to permanent male infertility. Three-dimensional(3D) in-vitro culture can operate as an effective strategy for studies on spermatogenesis and male infertility treatment. Sperm-like cells derived from the human and mice spermatogonial stem cells after frozen-thawed has already been carried out by researchers. In this study, 3D culture of testicular tissue by GF were studied in 4 groups of immature mice testes after 8 weeks to evaluate the progress of the spermatogenesis process.

Study design, size, duration: The testes were removed from 5 NMRI neonatal male mice (six days old) and repeated 3 times for further accuracy in each group. The testis tissue fragments were transferred to the hexahedrons, incubated in a culture incubator and cultured for 8 weeks
Participants/materials, setting, methods: The fragments of testes tissue are placed in the freezing culture. The tissue was examined from the aspect of morphology.PLZF, SCP3, ACRBP antibodies were assessed to identify spermatogonia, spermatocyte and sperm-like cell respectively. Four studied groups are as follow: Culture of fresh testicular tissue fragments on agarose with and without growth factors for 8 weeks ,the culture of frozen-thawed neonatal mouse testicular tissue fragments on the agarose with and without growth factors for 8 weeks.
Main results and the role of chance: The findings showed that the size and diameter of seminiferous tubules were increased compared to control groups. Different types of germ cells including spermatogonia and spermatocyte cells were observed in Fresh and frozen-thawed testicular tissue.
The expression level of ACRBP was significantly increased in the fresh culture group with growth factors and in the frozen-thawed culture group with growth factors (P≤0.05).
Limitations, reasons for caution: Further studies are needed, where the culture medium enriched with several supplements or growth factors, for better results.
Wider implications of the findings: The slow programmed freeze of the 3D agarose testicular tissue culture appears to be a useful method to maintain long-term preservation, especially for children with cancer before initiating treatment with chemotherapy or radiotherapy.These results will encourage researchers to set up a culture system for in vitro spermatogenesis.
Trial registration number: not applicable

P-422 Translating male cancer patients' needs into a professional development intervention that improved fertility preservation knowledge and referral among oncology healthcare providers

W.M. Buckett, M.B.B.Ch.-M.D.[1], B. Herrero[2], G. Raskovic[3], M.C. Brabant[4], P. Chan[5]

[1]MUHC Reproductive Centre, Obstetrics and Gynecology- McGill University, Montreal, Canada ;
[2]MUHC, Obstetrics and Gynaecology, Montreal, Canada ;
[3]MUHC, Oncology, Montreal, Canada ;
[4]MUHC, Obstetrics and Gynecology, Montreal, Canada ;
[5]MUHC, Urology, Montreal, Canada

Study question: How do male cancer patients want to learn about fertility preservation and how can this need be addressed improving care and referral rates?
Summary answer: Cancer patients perceived their oncology healthcare providers as the most trusted source from fertility preservation. Targeted seminars for oncology healthcare professionals improved knowledge and referral.
What is known already: Clinical practice guidelines for fertility preservation recommend clinicians to discuss the potential impact of cancer treatments on future fertility with reproductive-aged cancer patients, and be prepared to refer them to a reproductive specialist, if appropriate. However, multiple publications consistently show that pre-treatment fertility counselling is disseminated to only a minority of newly diagnosed cancer patients. Effective and co-ordinated strategies to overcome this and improve quality of life after cancer are urgently needed. One barrier is the lack of understanding of cancer-related infertility and available resources amongst both patients and their oncology practitioners.
Study design, size, duration: Co-ordinated and targeted regional strategy comprising: (1) Cancer patient surveys (March-July 2016) to determine fertility preservation needs; (2) Assessment of efficacy of educational seminars to oncology health care professionals (January 2017-December 2018); and (3) Evaluation of discussion of the effect of cancer care on subsequent fertility in men aged under 50 years and, where appropriate, referral rates (January 2018-July 2019)
Participants/materials, setting, methods: Male cancer patients (n=45) completed surveys about cancer and fertility and to choose most trusted sources for information - oncology healthcare professional (HCP); other HCP; peers; educational material; websites. Seminars were given at 7 different regional sites. Oncology HCPs (n=109) completed a pre- and post-surveys and differences were analyzed. Targeted chart review of men with a new diagnosis of cancer under 50 was undertaken to evaluate fertility preservation discussion and overall referral rates were measured.
Main results and the role of chance: The median age of patient respondents was 30 years and 69% were in a relationship. The majority (89%) of patients perceived oncology HCPs as the most trusted source to learn about fertility preservation and 11% preferred to learn from another channel of information (videos, brochures, websites). Following the survey and in order to fulfill the need expressed by respondents, a series of seminars were organized to bring awareness and knowledge to healthcare providers about fertility preservation and how to access the service. The mean pre-session knowledge score was 2.08, 2.46 and 2.06 for nurses, physicians and allied HCPs respectively, whereas the mean post-session knowledge score was 3.73 ($p<0.05$) for all. Documented evidence of discussion was 15% and referral rate for fertility preservation was 7.7% (104/1345) pre-intervention. The discussion rate was 35% and the referral rate 21% (379/1807) post-intervention ($p<0.001$). The reasons for non-discussion and non-referral were assessed.
Limitations, reasons for caution: The study samples size are small, and the fact that the seminars were performed in university-based hospitals limit the generalization of these findings. Furthermore, the evaluation of discussion and referral rates overlap the period of the educational intervention.
Wider implications of the findings: Oncology HCPs are perceived as the most trusted source of health information and advice among young male adults with cancer. Therefore, increasing knowledge of healthcare providers in fertility preservation could enable proactive and open discussions with patients, thus enhancing the quality of counselling and improving access to care.
Trial registration number: Not applicable

P-423 Oncofertility cares provided to patients: results from a systematic review of literature revealed a low adhesion to fertility preservation services due to accessibility barriers

J. Daolio[1], L. De Panfilis[2], M. Perin[2], M.C. Bassi[3], L. Mangone[4], M.T. Villani[1], A. Nicoli[1]

[1]Azienda Unità Sanitaria Locale - IRCCS di Reggio Emilia, Departement of Obstetrics & Gynecology, Reggio Emilia, Italy ;
[2]Azienda Unità Sanitaria Locale - IRCCS di Reggio Emilia, Unit of Bioethics, Reggio Emilia, Italy ;
[3]Azienda Unità Sanitaria Locale - IRCCS di Reggio Emilia, Medical Library, Reggio Emilia, Italy ;
[4]Azienda Unità Sanitaria Locale - IRCCS di Reggio Emilia, Unit of Epidemiology, Reggio Emilia, Italy

Study question: What kind of barriers could hinder the use of fertility preservation services?
Summary answer: Barriers in knowledge, attitude and organization of oncofertility cares could be present at patient, health providers and organizational levels limiting the adherence to service.
What is known already: International guidelines on oncofertility cares recommend to counsel patients of childbearing age about the risk of infertility following cancer treatment and options available to preserve fertility. Patients without fertility preservation choices before treatments have negative psychological consequences during the course of treatments and, in case of survival, on quality of life. Poor adherence to guidelines is acknowledged due to the presence of barriers in daily clinical practice regarding healthcare providers and information provided to patients hindering the access to fertility preservation services (FPS). In our realty, only 16,7% of patients were counseled for FPS between 2015-2017.
Study design, size, duration: This study was conducted from August 2019 to November 2019 as part of a major healthcare pathway about oncofertility services. In the first phase, we conducted a comprehensive systematic review of literature with the keywords "fertility preservation", "cancer", "education", "engagement"; in the second phase, results will be used by an expert team of Embryologists and Bioethicists to define educational interventions to overcome barriers in our network.
Participants/materials, setting, methods: The electronic search was conducted in Medline, Embase and Cinahl, and led to identify a set of 1540 items. The selection was confined to articles in English language only. Three authors screened individually all the items by title and abstract, and, after removing

duplicates and discussing about discrepancies, a selection of 320 articles was finally considered for the evaluation of contents.
Main results and the role of chance: The analysis by text revealed 4 key items on which the educational interventions could be focused: healthcare providers, patients, patient's engagement and implementation of educational FPS programs aimed at identifying criticisms on oncofertility decision-making counseling before treatments. Both healthcare providers and patients challenge with FPS barriers concerning communication, inappropriate support for a conscious decision-making process or lack of knowledge about fertility preservation strategies, etc. These represent core items on which building educational programs aimed to increase the discussion rate on FPS between clinicians and patients, by ensuring a personalized care path to patients in terms of time, use of available resources, quality and continuity of care, and a good quality of life. Suggested tools are the development of a multidisciplinary team, a script-based approach, the partners or relatives involvement at the time of counseling, the release of informative material and the training of professional figures working between oncologic and reproductive care services (nurses or midwives).
Limitations, reasons for caution: It is important to keep on sensitizing all multidisciplinary healthcare professionals involved in cancer care to avoid the underestimation of FPS barriers.
Wider implications of the findings: The evaluation of effectiveness of FPS awareness-raising interventions in terms of patient engagement can increase the number of FPS accesses, improve the knowledge of cancer therapies and the quality of the clinician/patient relationship. The implementation of multidisciplinary FPS programs can also increase the adherence to national guidelines on oncofertility cares.
Trial registration number: not applicable

P-424 In vitro nanoparticle-mediated delivery of MicroRNA 143 and MicroRNA 206 on spermatogonia and cancer cell apoptosis

A. Shams[1], M. Koruji[2], R. Shabani[3], M. Asghari[4], M. Karimi[5]

[1]Iran univesity of medical sciences, reproductive biology, tehran, Iran ;
[2]Iran univesity of medical sciences, anatomy, tehran, Iran ;
[3]iran university of medical sciences, anatomy, tehran, Iran ;
[4]Tabriz university of medical sciences, statistics, tabriz, Iran ;
[5]Iran univesity of medical sciences, nanotechnology, tehran, Iran

Study question: to assess the efficiency of PLGA-miR 143/206 transfection on apoptosis in mouse leukemia cancer cells (El4) and spermatogonial stem cells (SSCs)
Summary answer: we were able to induce apoptosis on cancer cells without any significant damage to spermatogonial stem cell by using smart transfection.
What is known already: A promising method to preserve fertility in children with cancer is via testicular biopsy before the onset of cancer treatment, followed by isolation, proliferation, maintenance and transplantation. One of the methods for the decontamination of cancerous cells from testicular cell suspensions can be smart nanoparticles. Smart gene delivery via Nanoparticles can be targeted and concurrently minimize damage to healthy cells in cancer treatments.Many microRNAs (MiRs) are used to induce apoptosis in cancer cells.Many studies have shown the effect of MiRs 143 and 206 on induction of apoptosis in many cancer cells. ransfection of MiRs via nanoparticles has been very promising.
Study design, size, duration: This study was in vitro animal experiment and it was done on 50 neonate mouse. This study was conducted over a period of 9 months
Participants/materials, setting, methods: mouse spermatogonia and cancer cell were used. To obtain a suitable miR dose that can induce apoptosis in cancer cells, while not harming SSCs, several doses were evaluated. Cells were treated separately at 3 doses of each miR (for miR 143, doses of 25, 50 and 75 nmol and for miR 206, doses of 50, 100 and 150 nmol) at 24, 48 and 72 hours. Viability and apoptosis were investigated by MTT and Annexin Kits
Main results and the role of chance: Based on MTT assay results, the optimal dose of miR 143 was 75 nmol (61.24%±2.85 SSC and 45.57%±0.78 EL4) (P≤0.05), and for miR 206, the optimal dose was 150 nmol (53.82%±6.7 SSC and 28.14%±3.01 EL4) (P≤0.05). The optimal time was 48h. these doses, the survival rate of the EL4 cells was below IC50 and SSC survival was above 50%. Annexin V staining also confirmed the selected doses (for miR 143 total apoptosis was 6.93%±2.8 SSC and 38.2%±13.2 EL4 (P≤0.05), and miR 206 was (14.6%±5.5 SSC and 38.3%±23.7 EL4) (P≤0.05).
Limitations, reasons for caution: The biggest limitation of this study was the failure to perform it on human specimens
Wider implications of the findings: Taken together, this study suggests that MiR -therapy may lead to the development of novel therapeutic strategies for cancer, and apoptotic MiRs may be a potential therapeutic agent for human tumors and is worthy of further investigation.
Trial registration number: 96-01-30-29861

P-425 Does letrozole supplementation during ovarian stimulation for fertility preservation impact early luteal progesterone levels following GnRH agonist trigger

I. Lalami[1], M. Peigné[1], C. Vinolas[2], F. Krief[2], I. Cédrin[1], M. Grynberg[1]

[1]Hôpital Jean Verdier, Department of Reproductive Medicine and Fertility Preservation, Bondy, France ;
[2]Hôpital Jean Verdier, Department of Reproductive Medicine and Fertility Preservation-, Bondy, France

Study question: To investigate early luteal progesterone levels following GnRH agonist (GnRHa) trigger after controlled ovarian stimulation (COS) in cancer patients with or without letrozole supplementation (COSTLES).
Summary answer: Following GnRHa trigger, early luteal phase progesterone levels are significantly lower in COSTLES when compared with stimulation performed without aromatase inhibitor.
What is known already: Oocyte vitrification after COS is the first line and most effective method for fertility preservation (FP) prior to cancer treatment. However, COS leads to supraphysiologic serum estradiol levels that could be potentially harmful in estrogen-sensitive diseases such as breast cancer. To protect patients from the potential adverse effects of elevated estradiol levels during COS, specific FP protocols using aromatase inhibitors (letrozole) have been developed recently. However, data on luteal progesterone levels following COS are lacking despite rising evidence of the role of progesterone in breast tumorigenesis.
Study design, size, duration: This retrospective study was conducted from July 2014 to December 2019. Serum progesterone levels following GnRHa trigger measured in 84 breast cancer patients undergoing COSTLES were compared to those obtained in 162 FP patients stimulated without letrozole administration.
Participants/materials, setting, methods: This study was performed in a public tertiary hospital. All women underwent COS with GnRH antagonist protocol. In COSTLES, the aromatase inhibitor was started 2 days before recombinant FSH administration and continued until GnRHa trigger. Final oocyte maturation was induced by injection of 0.2 mg of triptorelin, 36 hours before oocyte pick-up in both groups of patients. Serum hormonal levels of estradiol, LH and progesterone were measured in the morning following the injection of triptorelin.
Main results and the role of chance: Patients in COS without letrozole and COSTLES groups were comparable in terms of age (31.7±0.4 vs. 32.7±0.5, respectively), BMI (23.6±0.5 vs. 23.4±0.4 kg/m²), antral follicle count (22.2±1.0 vs. 22±1.7 follicles) and serum anti-Müllerian hormone levels (3.1±0.2 vs. 2.6±0.8 ng/mL). They received comparable total amount of exogenous FSH (3167.3±251.9 vs. 2981.2±237.2 IU). As expected, on the day following GnRHa administration serum estradiol levels were lower in COSTLES cycles (650.3±57.7 vs. 2451.4.0±144.0 pg/mL, p<0.01). However, GnRHa-induced LH surge was significantly higher in patients having received aromatase inhibitors (71.9±4.6 vs. 51.2±2.6 UI/L, p<0.01). In addition, serum progesterone levels were significantly lower in the COSTLES group (8.6 ± 0.7 vs. 10.5 ± 0.5 ng/mL, p< 0.03). Otherwise, the mean number of oocytes recovered (14.2±0.7 vs. 14.1±0.9 oocytes, respectively) and vitrified at metaphase 2 stage (10.1±0.6 vs. 10.0±0.7 oocytes) did not differ significantly between COS without letrozole and COSTLES.
Limitations, reasons for caution: Serum progesterone levels were available only once, during the very early luteal phase.
Wider implications of the findings: The higher LH surge following GnRHa administration in COSTLES protocol may be in relation with a weaker negative

feed-back of estradiol on the hypothalamic-pituitary axis. However, the lower serum progesterone levels might suggest a suppressive effect of letrozole in its production. Further analysis is required to confirm the mecanisms.
Trial registration number: not applicable

P-426 Gonadotrophin stimulation and risk of relapse in breast cancer

A. Fredriksson[1], E. Rosenberg[2], Z. Einbeigi[3], C. Bergh[1], A. Strandell[1]

[1]Sahlgrenska University Hospital, Reproductive Medicine, Gothenburg, Sweden ; [2]Sahlgrenska University Hospital, Gynaecology, Gothenburg, Sweden ; [3]Southern Älvsborg Hospital, Department of Medicine- Oncology, Borås, Sweden

Study question: Is gonadotrophin stimulation as part of IVF associated with an increased risk of relapse in breast cancer?
Summary answer: Controlled ovarian hyperstimulation (COH) in connection with IVF in women with previous breast cancer was not associated with an increased risk of breast cancer relapse.
What is known already: Breast cancer is the most common malignancy among women in the world and the leading cause of cancer death among females. The use of COH with gonadotrophins in order to rescue the fertility by cryopreservation of oocytes or embryos prior to cancer treatment is currently the most established fertility preservation method for women with breast cancer. To date, there are only a few small retrospective hospital-based controlled studies evaluating the risk of breast cancer relapse in patients undergoing fertility preservation with or without COH showing no evident risk of relapse in breast cancer after the use of chemotoxic agents.
Study design, size, duration: This was a retrospective, population-based, matched cohort study comprising a total of 5857 women, previously treated for breast cancer of whom 337 were exposed to COH. Exposure for COH and outcomes as relapse and death were identified for all patients from 2003 to 2014 by assessing national registries holding data on IVF, births, prescription of drugs, causes of death and cancer diagnoses. Relapse, when non-registered, was calculated based on typical diagnoses and procedures, indicating relapse.
Participants/materials, setting, methods: Women aged 20-44 years, previously diagnosed with breast cancer and exposed to COH, were matched for age at breast cancer diagnosis +/- five years, tumour size and lymph node involvement to a non-exposed control group. In a subsequent secondary analysis the entire cohort was assessed with adjustments for N- and T-stages.

The risk of r2elapse in breast cancer was estimated as crude hazard ratios (HRs) and 95% confidence intervals using Cox proportional hazards models.
Main results and the role of chance: In the matched cohort relapse occurred in 27 of 337 (8.0%) women having undergone COH compared with 71 of 334 (21.3%) among the non-exposed (HR=0.60; 95% CI 0.37-1.00; p=0.051). In the secondary analysis with adjustments for T- and N stages the risk remained low after COH exposure; 27/337 (8%) in the exposed cohort compared with 1176/5520 (21.3%) in the control group (HR=0.54; 95% CI 0.34-0.84; p=0.006). In the exposed cohort nine breast cancer related deaths (9/27; 8%) occurred compared with 555 in the non-exposed control group (555/5220; 10.1%). The coding template for assessing relapse from diagnoses and procedures in regions where relapse was not a registered variable, compared with a reference region, resulted in a sensitivity of 95% and specificity of 87%.
Limitations, reasons for caution: A substantial degree of missing data on important prognostic variables was a limitation. Further, data on confounding factors were not completely covered. Another limitation was that the variable 'relapse' had been introduced only in one region and had to be calculated from other variables.
Wider implications of the findings: In this large, retrospective, matched cohort study we found no risk of relapse in breast cancer among women who had been exposed to gonadotrophins as part of IVF. This is reassuring but probably confounded by selection to IVF of a group of women with a more favourable prognosis.
Trial registration number: Not applicable

P-427 Clustering of primordial follicles during reproductive ageing

A. Schenck[1], G. Greggains[1], M.V. Rodriguez[1], B. Davidson[2], P. Fedorcsak[1]

[1]Oslo University Hospital, Department of Reproductive Medicine, Oslo, Norway ; [2]Oslo University Hospital, Department of Pathology, Oslo, Norway

Study question: What is the spatial distribution of primordial follicles the human ovarian cortex during the reproductive lifespan?
Summary answer: Follicles lie clustered in the human ovarian cortex; average cluster radius is ~270µm. At older age, degree of clustering increases and the cluster size decreases.
What is known already: In mice, the distribution of follicles is clustered, and the degree of clustering is increased in aged mice. In human, primordial follicles distribute unevenly in the ovarian cortex, but the pattern of distribution has been unknown.
Study design, size, duration: Observational study using ovarian tissue samples derived from patients undergoing fertility preservation treatment at an academic hospital.
Participants/materials, setting, methods: Ovarian tissue samples derived from 14 patients (aged 20.8 – 35.2, mean 28.1 years) were obtained after unilateral oophorectomy. Cortical fragments were processed for quantitative histological assessment, including recording of two-dimensional coordinates of primordial follicles and stage of follicle development. The spatial distribution of follicles was tested and cluster radius was calculated. Loss of follicles was modeled with computer simulation, comparing alternative models of follicle loss dynamics.
Main results and the role of chance: Primordial follicles form clusters with a radius of ~270 µm in the ovarian cortex, which is increasingly apparent with ageing. At older age, follicle density declines and the distance to the nearest neighbouring follicle increases, the cluster radius decreases, but the degree of clustering increases. Computer simulation of follicle loss dynamics indicate the close-range follicle-to-follicle signalling may contribute to the emergence of clusters during reproductive ageing.
Limitations, reasons for caution: The tissue was obtained for diagnostic purposes. The small size of the fragments may limit the validity of assumptions of the spatial statistical analysis. All tissue samples were sectioned in the same orientation, limiting comprehensive three-dimensional analysis.
Wider implications of the findings: Clustering may impede precise estimation of total follicle count, the imprecision increasing with age. Excessive dissection of ovarian tissue fragments on ~ 0.5 mm scale may disrupt clusters and interfere with close-range follicle-to-follicle signalling, causing rapid graft exhaustion.
Trial registration number: not applicable

P-428 Semen quality and cryopreservation in patients with brain tumors.

D. Rivet-danon[1], C. Jean[1], A.S. Gille[1], C. Chalas[1], C. Patrat[1], V. Drouineaud[1]

[1]Assistance Publique – Hôpitaux de Paris AP- HP Centre- Université de Paris, Service de Biologie de la Reproduction - CECOS, Paris, France

Study question: Do initial sperm parameters of a patient with brain tumor make cryopreservation possible?
Summary answer: Overall, initial mean sperm parameters are normal except for progressive motility in pineal region tumors and make possible cryopreservation in the vast majority of cases.
What is known already: Baseline data on semen quality and cryopreservation in patients presenting brain tumors are mainly based on small sample sizes and results of various studies are controversial. Melatonin is a neurohormone secreted by the pineal gland. Literature data show that melatonin affects secretion of both gonadotropins and testosterone, and can influence sperm quality. Moreover, men having reduced sperm motility present lower levels of melatonin in semen than patients with normal sperm parameters. Furthermore, in vitro addition of melatonin in semen shows an increase of sperm progressive motility.
Study design, size, duration: This was an 8 years retrospective and observational study including patients with brain tumors, referred for sperm cryopreservation before a potentially gonadotoxic treatment, to the Paris Cochin University Hospital sperm bank (Centre d'Etude et de Conservation des Œufs et du Sperme humains, CECOS). The location of brain tumors plus clinical and histological data were collected as well as sperm parameters before freezing and after thawing. Fertile sperm donors were recruited between 2014 and 2016.

Participants/materials, setting, methods: Two hundred and fourteen brain tumor patients aged 14-58 years old and 92 fertile sperm donors were included. Sperm parameters were analyzed before freezing and after thawing, according to the World Health Organization's (WHO) 2010 guidelines. Sperm parameters and the quality of sperm straws were compared on one part between patients with brain tumors, in particular according to the location of the tumor, and on other part between patients and fertile sperm donors.

Main results and the role of chance: Among the 214 patients, brain tumor location was recorded in 183 patients: pineal gland (26), hypothalamic-pituitary axis (7) and other periphery brain regions (150).

In all patients, each mean initial sperm parameter was normal according to the WHO 2010 criteria (total sperm count: 326.5 ± 433.2 x 10^6, sperm concentration: 95.9 ± 108.16 x 10^6/ml, progressive motility: 32.7 ± 14.8%), except sperm vitality which was low (53.2 ± 19.2%).

However, mean progressive motility (p<0.001), vitality (p<0.001), morphology (p=0.03) and number of motile spermatozoa per straw (NMSPS) (p=0.008) were significantly lower in brain tumor patients than in donors, respectively.

In pineal brain tumor patients, mean sperm progressive motility was low according to the WHO 2010 criteria (27.8 ± 13.4%) and significantly lower than in the case of hypothalamic-pituitary tumors (27.8% vs 45.0%; p=0.009). All mean sperm parameters of patients with pineal tumor were significantly reduced compared to those of fertile donors (total sperm count, p=0.02; sperm concentration, p=0.005; progressive motility, p<0.001; vitality, p<0.001; morphology, p=0.01; NMSPS, p=0.01, respectively).

Severe oligozoospermia (sperm concentration <1 x 10^6/ml) was significantly more frequent in patients with pineal tumor than in periphery locations (p=0.007).

More than 90% of patients could bank sperm regardless of tumor location.

Limitations, reasons for caution: The abstinence period was high in patients with brain tumors and could explain the low sperm vitality and motility observed. The disease itself and/or brain surgery and/or the associated altered general state in those patients probably influenced the long abstinence delay.

Wider implications of the findings: Patients with brain tumors showed suitable sperm parameter allowing cryopreservation in most cases, even in pineal gland tumor patients. Importantly, sperm straws could be used for Assisted Reproductive Technologies (ART) if necessary. Those results confirm the importance of sperm cryopreservation in those patients before a potentially high gonadotoxic treatment.

Trial registration number: not applicable

P-429 Fertility preservation outcomes among adolescent transgender boys before testosterone treatment compared with adolescent cisgender girls

H. Amir[1], A. Oren[2], E. Klochendler Frishman[3], O. Sapir[3], Y. Shufaro[3], F. Azem[1], A. Ben-Haroush[3]

[1]Tel Aviv Sourasky Medical Center, Racine IVF Unit- Lis Maternity Hospital, Tel Aviv, Israel ;
[2]Tel Aviv Sourasky Medical Center, Pediatric Endocrinology and Diabetes Unit- Dana-Dwek Children's Hospital, Tel Aviv, Israel ;
[3]Rabin Medical Center, IVF and Infertility Unit, Petah Tikva, Israel

Study question: What are the fertility preservation (FP) outcomes among adolescent transgender boys before testosterone treatment compared with adolescent cisgender girls?

Summary answer: FP outcomes from assisted reproductive technology (ART) among adolescent transgender boys are excellent compared to adolescent cisgender girls.

What is known already: ART enables young transgender men to have genetically related children and overcome gender-affiliation hormone (i.e. testosterone) therapy-related fertility impairment. Although medical FP outcomes in young cancer patients who preserve fertility before exposure to anticancer therapy are well known, there are no data on FP outcomes among transgender boys before initiating testosterone therapy.

Study design, size, duration: This retrospective cohort study included 11 adolescent transgender boys and 39 adolescent cisgender girls who underwent FP between January 2017 and April 2019 and September 2013 and April 2019, respectively.

Participants/materials, setting, methods: Eleven transgender boys (mean age 16.4y) and 39 cisgender girls (mean age 15.5y) were referred to FP at IVF units of two tertiary university-affiliated medical centers. The transgender boys (study group) were referred before initiating testosterone treatment and the cisgender girls (control group) were referred due to cancer diagnosis before starting anticancer treatment. Control ovarian stimulation was carried out by the GnRH antagonist protocol. Statistical analyses compared ART data and FP outcomes between two groups.

Main results and the role of chance: The values of the accepted ovarian reserve markers, follicle-stimulating hormone (FSH: 5.7±1.9 mIU/mL) and antral follicle count (AFC: 20.4±5.5) of all the adolescent transgender boys were within the normal range. The transgender boys were slightly older compared to the cisgender girls (16.4±1 vs 15.5±1.3 years, respectively, $P = 0.03$). The amount of FSH used to stimulate the ovaries was significantly lower among the transgender boys compared to the cisgender girls (2308±945 IU vs 4372±1877 IU, respectively, $P < 0.001$), but the duration of ovarian stimulation was similar for both groups (12.2±3.6 and 10.1±2.8 days, respectively, $P = 0.092$). There were no significant differences between the transgender boys and cisgender girls in peak estradiol levels (2660±2532 pg/mL vs 1269±975 pg/mL, respectively, $P = 0.053$), the number of retrieved oocytes (30.5±11.4 vs 22±13.2, respectively, $P = 0.062$), the number of MII oocytes (24.9±11.7 vs 18.8±11.2, respectively, $P = 0.122$), and the maturity rates (80±10.5% vs 85.4±14.6%, respectively, $P = 0.118$).

Limitations, reasons for caution: Our control group consisted of oncology patients in whom the ovarian reserve and the ovarian stimulation response may be affected by the underlying disease and it may, therefore, not be representative of the general healthy population.

Wider implications of the findings: Adolescent transgender boys have a normal ovarian reserve and an excellent response to ovulation stimulation before initiating testosterone treatment. Oocyte cryopreservation is, therefore, a feasible and effective way for them to preserve their fertility for future biological parenting.

Trial registration number: Not required

P-430 Passive slow freezing is an easy, cost-effective and efficacious alternative freezing method for slow controlled cryopreservation of ovarian tissue.

S. Lierman[1], A. Bus[2], S. Andries[2], P.E.J. Bols[2], K. Tilleman[1]

[1]University Hospital Ghent- Fertility and Stem cell Team, Department for Reproductive Medicine, Ghent, Belgium ;
[2]Laboratory for Veterinary Physiology and Biochemistry- Gamete Research Centre, Faculty of Pharmaceutical Biomedical and Vetrinary Science- University of Antwerp, Antwerp, Belgium

Study question: Is there a difference in follicle activation after xenotransplantation of passive slow frozen ovarian tissue compared to standard controlled frozen tissue?

Summary answer: Human primordial follicles have similar developmental capacity in a xenotransplantation model after passive slow rate freezing (PSF) compared to the control slow rate freezing (CSF).

What is known already: For the moment, CSF is the established method for the freezing of cortical fragments of ovarian tissue (OT). CSF requires expensive computerized equipment and the process is time-consuming. There is a risk for technical failures with the potential loss of precious material. Other techniques like tissue vitrification are promising. However, the technique is expensive and laborious. PSF is a technique that is successfully used in freezing of in vitro cell cultures and testicular tissue of different animal species including human (Goossens et al., 2013). Until today no studies have described PSF for human ovarian tissue.

Study design, size, duration: Xenotransplantation was performed in the pouch between the musculus obliquus and peritoneum of Balbc/nude mice (n=24). On each side of the incision at the linea alba, a PSF and a CSF frozen-thawed OT-piece of the same patient was introduced. After 2,4 and 6 weeks transplantation, mice were euthanized by cervical dislocation and grafts were collected, fixed in 4% buffered formalin and embedded in paraffin for HE staining. All xenotransplantation experiments were performed in duplo.

Participants/materials, setting, methods: In total, 48 OT strips from 4 transgender patients were frozen using L-15+0.45% HSA+DMSO (1.5M). For

CSF a programmable freezer was used (2°C/min:4°C to –9°C; seeding at –9°C; –0.3°C/min to –40°C and to –140°C by 10° C/min). PSF was performed using an isopropyl alcohol container (Mr. Frosty, Nalgene) and stored at –80 °C overnight at ≈ -1°C/min. Follicle activation and the fibrotic surface area by Masson Trichrome staining were analyzed. Fisher's exact-test was performed.

Main results and the role of chance: The grafting recovery rate was 95.8% (46/48 OT-strips). Both freezing procedures, PSF versus CSF, showed similar amounts of primordial follicles after 2 hours of warming before transplantation (53.0% (1690/3188) versus 54.7% (1309/2393) (P=0.220)).

After 2 weeks of transplantation, a clear and significant activation of primordial follicles in both cryopreservation methods was seen compared to the non-transplanted tissue. For PSF (85.3% (290/340) versus 47.0% (1498/3188) and CSF (72.7% (288/396) versus 45.3% (1084/2393), (P<.0001).This activation shift was significantly higher in PSF compared to CSF (85.3% (290/340) versus 72.7% (288/396) (P<.0001). After 4 weeks, the PSF technique showed a higher percentage of secondary follicles in the growing follicle pool in comparison to CSF 37.6% (65/173) versus 6.8% (4/59) (P<0.001).

After 6 weeks, the proportion of secondary follicles was not significantly different between the two methods (CSF 47.5% (19/40) versus PSF 38.5% (15/39)).

The percentage of fibrosis in the xenograft was comparable for PSF versus CSF for the 2 and 4 weeks (10.25±0.37 versus 10.50±0.37) and (9.42±0.47 versus 10.02±0.37). After 6 weeks transplantation, graft tissue from the PSF showed a higher percentage fibrotic area compared to the tissue of the CSF (13.56±0.48 versus 11.55±0.46, (P=0.003)).

Limitations, reasons for caution: Although the follicle activation and growth in xeno transplanted human OT after PSF is similar to CSF, PSF has not yet been used in transplantation in human. The true competence of the PSF frozen tissue still needs proof of concept in human.

Wider implications of the findings: Passive slow freezing (PSF) shows a significant higher follicle activation after xenotransplantation than the conventional method of freezing (CSF). PSF could be an easy and cost-effective alternative to CSF for fertility preservation of human ovarian tissue.

Trial registration number: This research is conducted with the approval of the local ethics committee 2015/0124 – B670201523543), Ethical Committee on Animal Experimentation (ECD18/12).

P-431 Early follicle activation, repair and death after human ovarian tissue transplantation using adipose tissue-derived stem cells

L. Cacciottola[1], G. Courtoy[2], C. Hossay[1], J. Donnez[3], M.M. Dolmans[1]

[1]Institut de Recherche Expérimentale et Clinique- Université Catholique de Louvain, Gynecology Research Unit, Brussels, Belgium ;
[2]Institut de Recherche Expérimentale et Clinique- Université Catholique de Louvain, Imaging Platform, Brussels, Belgium ;
[3]Society for Research into Infertility, Av. Grandchamp 143, Brussels, Belgium

Study question: Does ovarian tissue transplantation (OTT) using adipose tissue-derived stem cells (ASCs) yield better follicle outcomes shortly after transplantation than the standard procedure?

Summary answer: Improved survival, reduced activation and better maintenance of repair mechanisms were observed in primordial follicles 3 days after grafting using ASCs compared to standard OTT.

What is known already: OTT has gained ground as a valid fertility restoration approach thanks to its established effectiveness. Nevertheless, more than 50% of follicles are lost after transplantation due to two main mechanisms: prolonged hypoxia and massive follicle activation, the latter known as the 'burn out' effect. ASCs have been shown to shorten the hypoxic/ischemic period by boosting revascularization in human xenografts, thereby increasing follicle survival. It is also known that hypoxia-related signaling is able to modulate several pathways involved in cell survival and follicle activation, like the PI3K/pAKT and Hippo pathways.

Study design, size, duration: Twenty nude mice (Swiss Nu/Nu) were grafted with frozen-thawed ovarian fragments from 5 patients. Ten of them underwent OTT in 2 steps using ASCs, as previously described by Manavella et al. (ASCs+OT group), and the remaining ten using the standard procedure (OT group). In each group, grafts were retrieved on days 3 (n=5) and 10 (n=5). One fragment was taken from each patient to serve as non-grafted controls.

Participants/materials, setting, methods: Prospective experimental study conducted in an academic gynecology research laboratory.

Retrieved xenografts were analyzed by histology (follicle count and classification), immunohistochemistry (caspase-3 for apoptosis and LC3B for autophagy) and immunofluorescence (analysis of follicle activation pathways): (i) FOX01 cytoplasm translocation for PI3K/pAkt pathway activation; and (ii) YAP nuclear translocation for Hippo pathway activation. Immunostained cellular localizations were determined in primordial follicles by image acquisition using the Axio Imager with ApoTome at 63x magnification.

Main results and the role of chance: The ASCs+OT group showed significantly higher follicle density and lower follicle atresia than the OT group over time. Nevertheless, ASCs did not appear to modulate apoptosis, as the rate of caspase-3+ follicles was higher in both groups after 3 days compared to non-grafted controls (2-step/ASCs+OT group: 24.3 %; OT group: 25%; non-grafted controls: 3.75%; p<0.05). Regarding autophagy, average LC3B staining concentrations were similar in the ASCs+OT group and non-grafted controls, but significantly lower in primordial follicles in the OT group, indicating less active cell repair.

FOX01 localization was mainly nuclear in oocytes of primordial follicles in non-grafted controls. A significant shift to cytoplasmic localization (follicle activation marker) was observed in the OT group on day 3, while the ASC+OT group showed no difference from non-grafted controls.

YAP localization was largely cytoplasmic in granulosa cells of primordial follicles in non-grafted controls, but shifted significantly to nuclear (follicle activation marker) in both grafted groups 3 days after OTT. ASCs appeared to confer a protective effect on primordial follicles by suppressing the PI3K/pAKT pathway. However, there was no evidence of Hippo pathway modulation by ASCs.

Limitations, reasons for caution: These findings warrant long-term studies investigating maintenance of the primordial follicle pool over time, with a view to clinical application in patients undergoing OTT.

Wider implications of the findings: The present study provides further insights into the mechanisms of action involved in follicle activation and cell repair, underlining the beneficial impact of ASCs in grafted ovarian tissue. We also demonstrated that ASCs exert positive effects on the ovarian reserve by protecting primordial follicles.

Trial registration number: NA

P-432 TurnerFertility study: preliminary data on fertility preservation by ovarian tissue cryopreservation in young girls with Turner syndrome

S. Nadesapillai[1], M. Schleedoorn[1], R. Peek[1], D. Braat[1], J. Van der Velden[2], K. Fleischer[1]

[1]Radboud University Medical Center, Obstetrics and Gynaecology, Nijmegen, The Netherlands ;
[2]Radboud University Medical Center, Pediatric Endocrinology, Nijmegen, The Netherlands

Study question: Which girls with Turner syndrome (TS) could benefit from fertility preservation by ovarian tissue cryopreservation (OTC), based on the presence of follicles in relation to karyotype, clinical and hormonal data?

Summary answer: Girls with TS who have favourable predictive parameters (e.g 46,XX cell line, a measurable AMH or spontaneous puberty) could benefit from fertility preservation by OTC.

What is known already: Infertility due to premature ovarian insufficiency is a major concern for girls with TS and their parents. Physicians are often asked about possible options to preserve their fertility. However, evidence for successful fertility preservation by OTC/autotransplantation in these girls is lacking and many questions remain. Without evidence on the effectiveness of OTC it cannot routinely be offered to girls with TS.

Study design, size, duration: A national prospective exploratory intervention study. Ovarian cortex will be obtained after unilateral ovariectomy from 100 girls with TS aged 2-18 years. Patients will be included between 2017 and 2020.

Participants/materials, setting, methods: All girls with TS who have completed the diagnostic work up of TS were included. After unilateral ovariectomy, one fragment of the ovarian cortex was used to determine the number of follicles by serial sectioning and staining. Karyotyping of ovarian cells, lymphocytes, buccal

cells and urine cells was performed by Fluorescence in situ hybridization (FISH). Blood samples obtained before ovariectomy and during the yearly clinical visit after ovariectomy will provide information on hormonal parameters.

Main results and the role of chance: Currently, we have received 98 informed consent forms and 66 girls (age 3-19) had an unilateral ovariectomy. Oocytes were found in 27% of the girls (n=18; age 5-17). In this group, 5 girls were prepubertal, 12 girls had a spontaneous thelarche (age 10-17) and 4 girls had a spontaneous menarche (age 13-16). Hormone values and the chromosome pattern were found to be predictive parameters for the presence of follicles. In this cohort, the chance of finding follicles in girls with a 46,XX cell line is three times higher than in girls without a 46,XX cell line. Until now, there was only 1 girl with 45,X cells in lymphocytes and buccal cells (n=25) who had follicles and a measurable AMH. FISH was used to karyotype the ovarian cortex cells of 5 patients with a mosaicism and revealed that 42 of the 46 oocytes that were analysed had a normal X-chromosomal content. Granulosa cells were largely 45,X, but showed different levels of X chromosome mosaicism, not only between patients but also between individual follicles of the same patient. Despite the presence of a low percentage (10–45%) of 46,XX ovarian stromal cells, normal macroscopic ovarian morphology was observed.

Limitations, reasons for caution: The final analysis will be performed when the dataset of 100 TS girls is completed. Further research is necessary to determine the functionality of the follicles in the ovaries of TS girls and to elucidate if OTC is an effective method for fertility preservation in TS.

Wider implications of the findings: A combination of clinical, hormonal and karyotypic data could provide predictive parameters to define which girls with TS might benefit from fertility preservation. These parameters could help physicians during fertility preservation counselling to determine if OTC is an option for a certain girl with TS.

Trial registration number: NCT03381300

P-433 Experience with 54 cryopreserved ovarian tissue grafts in a single centre: the devil in reporting details!

G. Rozen[1], F. Agresta[2], D. Gook[1], S. Siii[3], K. Stern[1]

[1]Royal Women's Hospital and Melbourne IVF, Reproductive Services, Melbourne, Australia ;
[2]Melbourne IVF, Reproductive clinic, Melbourne, Australia ;
[3]South West Healthcare- 25 Ryot Street- Warrnambool- 3280, Obstetrics and Gynaecology, Victoria, Australia

Study question: Following transplantation of cryopreserved ovarian tissue, what are IVF outcomes per cycle and are there factors related to this technique which impact on success.

Summary answer: Mature oocytes which resulted in pregnancies were largely harvested from non-ovarian grafts. Longer duration of ovarian activity correlated with increased tissue volume and follicle density.

What is known already: Transplantation of cryopreserved ovarian tissue has a unique role for fertility preservation and over 140 live births have now been published. Nevertheless, uncertainties remain regarding its efficacy, with success of this technique reported inconsistently and mostly reported per patient, rather than per cycle started or embryo transferred as is used in conventional IVF. This hampers identification and optimisation of factors which may predict success, such as grafting sites and tissue volumes.

Study design, size, duration: We analysed a case series of reproductive outcomes throughout the IVF treatment process following ovarian tissue transplantations at our centre (2006-2019), with tissue frozen in the years 1996-2018. This included 54 grafting procedures in 40 patients, following a diagnosis of premature ovarian insufficiency. Results of modified low-dose stimulation and oocyte retrieval, including both oocyte cryopreservation and IVF cycles were reviewed.

Participants/materials, setting, methods: Ovarian tissue was harvested from 40 patients at risk from gonadotoxic treatment at median age of 26 years (range 18-39). Almost 30% of patients had their tissue frozen elsewhere. Nine of 31 patients (29%) had prior cytotoxic treatment. Following a diagnosis of premature ovarian insufficiency, ovarian tissue was grafted to pelvic sites including the ovary and adjacent pelvic side walls, as well as the anterior abdominal wall, on average five years following extraction.

Main results and the role of chance: Fifty-four grafting procedures were performed in 40 women. For patients whose tissue was cryopreserved in our centre, ovarian activity resumed in 90% of grafts, and in 64% when cryopreserved elsewhere. This was after a mean period of 5.3 months. Follicle density and tissue volume grafted appear to be the strongest predictors of ovarian activity duration over 12 months, however this does not reach statistical significance. Stimulated cycles in 25 patients with low-dose modified recombinant FSH protocol from 2012, yielded an oocyte from 68% of follicles (32% rate of empty follicles) and 75% of these were retrieved from non-ovarian grafts. Mature oocytes were retrieved from mean follicular size 13.5mm (range 6-20 mm). The overall 2PN fertilisation rate was 64%. A total of 25 fresh and frozen embryos were transferred in 15 women, yielding a clinical pregnancy rate 28% (7/25) and live birth rate of 20% (5/25). There was additionally a single, ongoing spontaneous pregnancy. All pregnancies occurred in patients aged under 32 years at cryopreservation and only one of the successful patients had prior chemotherapy exposure.

Limitations, reasons for caution: Trends alone can be observed from this data as the numbers are small.

Wider implications of the findings: This case series adds to international understanding of this technique's results. It encourages detailed and consistent reporting, as ongoing research is needed to improve efficacy. These results support offering ovarian cryopreservation to young women who are undergoing fertility threatening treatment, with optimism for future pregnancy.

Trial registration number: not applicable

P-434 Dynamic in vitro culture of human ovarian cortical tissue improves follicle growth and the expression of genes involved in follicle activation and progression.

R. Talevi[1], M.M. Pallotta[1], V. Barbato[1], M. Di Nardo[1], V. Costanzo[1], R. Ferraro[2], G. Mondrone[3], K.S. Adiga[4], T. Capriglioine[1], G. Catapano[5], R. Gualtieri[1]

[1]Università degli studi di Napoli Federico II, Dipartimento di Biologia, Napoli, Italy ;
[2]Genesis Day Surgery, IVF center, Caserta, Italy ;
[3]IVF RED srl, research, Caserta, Italy ;
[4]Kasturba Medical College Manipal Academy of Higher Education, Clinical Embryology, Manipal, India ;
[5]Università della Calabria, Ingegneria Meccanica- Energetica e Gestionale, Rende CS, Italy

Study question: Does dynamic (D) versus static culture of human ovarian cortical tissue (HOCT) better support follicle growth and expression of genes involved in follicle activation and progression?

Summary answer: D vs static culture of HOCT improves follicle progression to the secondary stage and the expression of genes involved in follicle activation and progression

What is known already: Oxygen availability, disruption of solutes gradients and application of physiological fluid mechanical stress, through dynamic culture *in vitro* of HOCT have been shown to enhance follicle growth and health. Several pathways, e.g. Hippo, Wnt, and P3K/AKT, are modulated by fragmentation and subsequent culture of HOCT *in vitro*.

Study design, size, duration: Biopsies of HOCT were fragmented into 1×1×0.5mm strips and cultured, in groups of ten, in gas-permeable dishes (PD) and in D condition for 6 days. At the end of culture, the following endpoints were assessed: 1) follicle quality and stage; 2) follicle viability; 3) expression of genes involved in follicle activation and growth. This study was approved by our local ethical committee.

Participants/materials, setting, methods: Biopsies were obtained from five consenting patients (aged 27.2±5.8) during laparoscopic surgery for benign gynecological conditions, fragmented using a tissue chopper and cultured 6 days in PD and D conditions. Follicle health and stage were assessed through histology, and live-dead far-red/Hoechst 33342 labeling. Total RNA was extracted to analyze the expression of BMP15, BMP7 (Wnt pathway), INHA (follicle growth), FOXO3, FGF2 (P3K/AKT pathway), MST2, YAP1 (Hippo signaling).

Main results and the role of chance: Overall, 2300 follicles were analyzed. At day 0 most follicles were primordial (primordial, 78.7%; primary, 18.3%; secondary, 3%), with high viability (78.8%) and good quality (grade 1, 43.6%; grade 2, 29.2%; grade 3, 27.2%). At day 6, D was superior to PD culture in terms of follicle progression (primordial, 27.8 vs 40%; primary, 50 vs 45.3%; secondary, 22.2 vs 14.7%; $p<0.01$) and quality (grade 1, 43.6 vs 33.6%; grade 2, 27.4 vs 24.6%; grade 3, 29 vs 41.8%; $p<0.01$). Viability was not significantly different

between samples (day 0: 78.8%; PD: 67.9%; D: 65.3%). At day 6 of in vitro D versus PD culture, the expression of genes involved in follicle activation (FGF2; p<0.001) and progression (BMP7, p<0.05; BMP15, P<0.01) increased, whereas the expression of the inhibitors of cell proliferation (INHA, FOXO3; p<0.001) decreased. The expression of MST2 and YAP1, components of the Hippo pathway, was different between the two in vitro culture systems. In particular, MST2 was uP-regulated and YAP1 was down-regulated (p<0.05) after D versus PD culture.

Limitations, reasons for caution: This is an In vitro study carried on a limited number of patients

Wider implications of the findings: Dynamic vs static culture, which enhances the number and health of secondary follicles, and expression of genes involved in cell proliferation and follicle growth, could represent a new tool for in vitro folliculogenesis

Trial registration number: not applicable

P-435 Can progesterone primed ovarian stimulation (PPOS) be introduced in non-medical fertility preservation? Results from vitrified oocytes from the oocyte donation program.

M. Devesa Rodriguez De La Rua[1], N.P. Polyzos[1], E. Clua[1], D. Rodríguez[1], S. García[1], L. Coll[1], F. Martínez[1]

[1]Dexeus Mujer, Obstetrics- Gynecologý and Reproductive Medicine, Barcelona, Spain

Study question: Does the type of LH peak suppression (PPOS vs. GnRH antagonist) have any effect on oocyte competence, embryo development and live birth rates in recipients of vitrified donated oocytes?

Summary answer: PPOS protocol results in equally competent oocytes / embryos and comparable live birth rates as compared with GnRH antagonist (ganirelix) in recipients of vitrified oocytes.

What is known already: It has been reported that progesterone is effective in LH peak suppression in oocyte donation and IVF cycles, with no differences in the number of retrieved oocytes or embryo quality. Although some studies have found lower pregnancy rates in recipients of oocytes coming from a PPOS cycle suppressed with medroxyprogesterone acetate, results with desogestrel did not reveal significant differences. However, no study has analyzed results coming specifically from vitrified oocytes. Assessing outcomes from vitrified oocytes coming from donation cycles suppressed with progesterone in comparison to GnRH antagonist, would allow applying the same protocol to non-medical fertility preservation.

Study design, size, duration: Retrospective cohort study carried out from 2016 to 2018 in a University affiliated private fertility clinic.

Donors: age between 18-35 years, regular menstrual cycles, BMI 18-28 Kg/m^2 and no relevant medical history. LH suppression with desogestrel (PPOS): n=83. LH suppression with ganirelix: n=83.

Recipients: age <50 years, reception of vitrified oocytes coming from oocyte donation cycles suppressed either with desogestrel (n=83) or with ganirelix (n=83).

Participants/materials, setting, methods: Donors: pretreatment with oral contraceptives, stimulation carried out with gonadotrophins and downregulated with oral desogestrel (75mg daily, from stimulation day 1 until day of trigger) or subcutaneous ganirelix (0.25 mg/day, flexible protocol).

Recipients: endometrial preparation with oral estradiol valerate (2mg/8 hours) and vaginal progesterone (200 mg/8 hours). Embryo transfer was performed on third or fifth day of embryo development. Treatment was continued until the day of βhCG and, if positive, until 10th week of pregnancy.

Main results and the role of chance: Donors: There were no significant differences concerning mean age, BMI, AMH, AFC, number of mature (21.05 ± 7.52 vs. 23.05 ± 9.28) and vitrified oocytes (12.93 ± 6.66 vs. 15.17 ± 9.92) between desogestrel and ganirelix cycles, respectively.

Recipients: Age and BMI did not differ significantly between both groups of recipients. The number of thawed oocytes was significantly lower in the desogestrel group compared to the ganirelix group (9.89 ± 4.05 vs. 10.69 ± 3, p=0.02). Warming survival rates (80.1% vs. 81.1%), fertilization rates (71.3% vs. 75%) and embryo development rate (53.1% vs. 52.1%) didn't differ significantly between desogestrel and ganirelix groups, respectively.

Embryo transfer rate did not differ significantly between the groups (91.6% in desogestrel vs. 88% in ganirelix). Endometrial thickness and estradiol levels the day of fertilization showed no significant differences but progesterone levels were significantly higher in recipients of ganirelix protocol (8.19 ± 3.08 vs. 10.15 ± 7.05, p=0.03).

Mean number of transferred embryos showed no significant differences (1.29 ± 0.62 in desogestrel vs 1.13 ± 0.6 in ganirelix, p=0.09). No statistically significant differences were found in clinical pregnancy rates (48.2% in desogestrel vs. 53% in ganirelix) and live birth rates (37.3% in desogestrel vs. 43.4% in ganirelix).

Limitations, reasons for caution: Retrospective study carried out in oocyte donors that vitrified oocytes, which is a suitable model to suggest outcomes in non-medical fertility preservation, but the study did not analyze fertility preservation cycles specifically. Further well conducted clinical trials and long-term safety studies are needed.

Wider implications of the findings: According to our findings, no significant differences were observed in oocyte competence, embryo development and live birth rates from vitrified oocytes coming from desogestrel or ganirelix suppressed cycles. Therefore, non-medical fertility preservation cycles could be suppressed with progesterone instead of GnRH-antagonists, maintaining the efficacy, increasing patient convenience and decreasing costs.

Trial registration number: not applicable

P-436 Choosing a triggering agent in oocyte preservations cycles - A comparison between gonadotropin-releasing hormone agonist (GnRH-a) and recombinant human chorionic gonadotropin (r-hCG).

E. Haikin Herazberger[1], S. Knaneh[1], D.B.Y. Ben Yosef[1], H. Amir[1], A. Reches[1], Y. Kalma[1], F. Azem[1], N. Samara[1]

[1]Tel-Aviv university, IVF Unit- Department of Obstetrics and Gynecology- Tel-Aviv sourasky Medical centere- Tel-Aviv- Israel, Tel aviv, Israel

Study question: Is there a difference in outcomes of oocyte preservation cycles according to the type of triggering agent?

Summary answer: GnRH-a significantly increases the mature oocyte rate in medically indicated cycles, with no similar effect in social fertility preservation cycles.

What is known already: Fertility preservation is no longer reserved for patients with malignancy before medical intervention. More and more women seek to extend their fertility options due to personal choices. One main advantage of an antagonist protocol is that it provides the option to trigger ovulation with GnRH-a, which lowers the risk of ovarian hyperstimulation syndrome (OHSS). However, it also lowers pregnancy and live birth rates in fresh embryo transfer cycles due to an inadequate luteal phase. In fertility preservation luteal phase is irrelevant. We set out to compare other relevant outcomes according to triggering type in this subgroup of patients.

Study design, size, duration: This retrospective cohort study was performed at a tertiary university-affiliated medical center. Overall, 226 fertility preservation cycles between May 2013 and September 2018 were included in the analysis, of which 126 cycles were triggered with GnRH-a and 100 cycles with r-hCG. Medical indications accounted for 130 cycles and social indications accounted for 96 cycles.

Participants/materials, setting, methods: Fertility preservation indications were extension of fertility or fertility protection preceding chemotherapy, radiotherapy, or endometriosis surgery and fragile X carriers at risk for premature ovarian insufficiency. Triggering with GnRH-a (Decapeptyl 0.2 mg) or r-hCG (Ovidrelle 250 microgram) was administrated when the leading follicle was ≥17 mm with adequate estradiol levels. Mature oocyte rates were calculated as the number of MII oocytes/total retrieved oocytes per cycle. A cut-off of 80% indicated good oocyte maturation rate.

Main results and the role of chance: No significant differences were observed between patients triggered by GnRH-a compared to r-hCG in terms of gonadotropin dosage and BMI (p=0.95 and 0.62, respectively). The majority of patients with social indication were triggered with GnRH-a (63.5%). Patients with medical indications were divided equally between GnRH-a and r-hCG (p=0.043). The average age of the GnRH-a patients was significantly lower than that of the r-hCG patients (p<0.01), and the estradiol blood levels of the former were higher than those of the latter (p=0.04). There was no significant difference in oocyte maturation rate between triggering by GnRH-a or r-hCG (p= 0.48). Comparison of the percentage of GnRH-a- and r-hCG-triggered cycles with an MII rate >0.8 revealed a slightly higher percentage for the GnRH cycles, however,

difference was not statistically significant. (59.6% versus 47.1%, p=0.08). Data were then analyzed according to both indication for fertilization therapy and triggering agent. GnRH-a significantly increased the likelihood of a high maturation rate by 3.55 (1.30-9.66) among treatment cycles for medical indications, while there was no significant effect of the triggering agent in treatment cycles for social indication.

Limitations, reasons for caution: This cohort analysis is based on retrospective data collection, with potential biases inherent to that design. Pregnancy rates and live birth rates were not evaluated, and future studies on these aspects are warranted.

Wider implications of the findings: The results affirm that triggering with GnRH-a and r-hCG have similar ART outcomes in women planned to no fresh embryo transfer. GnRH-a triggering administration should be considered first choice in fertility preservation for reducing the risk of OHSS and for greater likelihood of good maturation rates in medically indicated cycles.

Trial registration number: not applicable

P-437 Epigenetic factor regulation; microRNA-9 and microRNA-218 related to cell proliferation and apoptosis, by tissue engineering-fibrin encapsulation: Preliminary study on ovarian tissue cryopreservation for fertility preservation

P. Thuwanut[1], W. Sereepapong[1], K. Pruksananonda[1], C. Suebthawinkul[1], P. Tuntiviriyapun[1], C. Taweepolcharoen[1], P. Sirayapiwat[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine- Chulalongkorn University, Bangkok, Thailand

Study question: Could fibrin encapsulation regulate alteration of epigenetic factors related to cell proliferation and apoptosis; microRNA-9 and microRNA-218, during ovarian tissue cryopreservation procedure?

Summary answer: The tissue engineering technology by fibrin encapsulation tended to down-regulate microRNA-218 (cell apoptosis) while uP-regulate microRNA-9 (cell proliferation) during ovarian tissue cryopreservation.

What is known already: Application of biodegradable matrix i.e. hydrogel or fibrin for mammalian cell encapsulation has been extensively considered one of potential strategies which offer utility of cell-based system regulation under complex microenvironment. Recently, this fascinating technology was introduced to reproductive medicine research i.e. immature testicular tissue fragment encapsulation or mesenchymal stem cell encapsulation in 3D ovarian cell construction. This novel approach allows molecule cocktails; angiogenic, growth factors, hormones or essential nutrients delivered to cell or tissue leading to cell/graft survival during culture, cryopreservation and transplantation. This technology, however, mainly presents in animal research whereas in terms of human ovarian studies are limited.

Study design, size, duration: The cortical pieces of ovarian tissues collected from two patients during July-August, 2019 (there cortical pieces per patient) were allocated into three sub-groups; I) fresh (F), II) ovarian tissue without fibrin gel encapsulation (Non-FG) and III) ovarian tissue encapsulated with 10% fibrin gel (FG). The cortical pieces from each designed group (Non-FG and FG) were processed for slow freezing procedure and evaluated for relative microRNA (microRNA-9 and microRNA-218) and mRNA (Bcl-2 and FOXO1) expression levels.

Participants/materials, setting, methods: Total of six cortical strips (0.5 x 0.5 x 1.0 mm; width x length x thickness) from premenopausal patients (age 39 and 48 years old; diagnosed with breast cancer and uterine fibroid) were allocated as mentioned in study design. Ovarian tissues were cryopreserved by -1°C/minute slow freezing (4°C to -80°C and plunged into -196°C). Fresh and post-thawed samples were analysed for relative microRNA (reference to U6) and mRNA (reference to GAPDH) expression levels using qRT-PCR.

Main results and the role of chance: The present findings preliminary indicated that the fresh ovarian cortical strips present the highest relative microRNA-9 expression level (2.97 ± 1.43; mean ± SD). Nonetheless, the lower relative microRNA-218 (2.06 ± 2.27; mean ± SD), Bcl-2 (4.42 ± 0.38; mean ± SD) and FOXO1 (7.23 ± 5.43; mean ± SD) expression levels compared the post-thawed samples were observed. Regarding to the recent advance in tissue engineering technology, the uP-regulation of microRNA related to cell proliferation (microRNA-9) was notably revealed in the post-thawed ovarian cortex encapsulated with fibrin gel which was 1.95 times higher than non-FG samples (FG = 1.13 ± 0.19 vs Non-FG = 0.58 ± 0.22; mean ± SD). Contrastingly, the genetic and epigenetic controls comprehended with form of programmed cell death; Bcl-2, FOXO1 and microRNA-218 were markedly lower or down-regulated in the ovarian tissue-fibrin encapsulated group (Bcl-2; Non-FG = 7.31 ± 1.52 and FG = 5.51 ± 1.16; FOXO1; Non-FG = 9.42 ± 3.01 and FG = 7.98 ± 0.43; microRNA-218; Non-FG = 2.39 ± 0.99 and FG = 0.63 ± 0.25; mean ± SD).

Limitations, reasons for caution: The present research findings preliminary document outcomes from a small number of participants following oophorectomy procedure. Additionally, the limitation of retrieved ovarian tissue size and number theoretically leads to the restriction of research or study design expansion and recapitulation in all aspects.

Wider implications of the findings: Our preliminary research outcomes speculate that the novel technology focused on tissue engineering with biodegradable gel matrix may be applicable for ovarian tissue cryopreservation and fertility preservation. Furthermore, the cortical striP-fibrin encapsulation may subsequently lead to the restriction of cell apoptosis during ovarian tissue cryopreservation and transplantation.

Trial registration number: 384/62

P-438 The use of LH for preserving reproductive health in cancer patients

S. Marcozzi[1], R. Vicenti[2], G. Orlando[3], C. Centonze[3], M. Macciocca[2], F. Di Rella[4], M. De Felici[1], R. Fabbri[2], F.G. Klinger[1]

[1]University of Rome Tor Vergata, Dep. of Biomedicine and Prevention, Rome, Italy ; [2]S.Orsola-Malpighi Hospital- University of Bologna, Department of Medical and Surgical Sciences DIMEC, Bologna, Italy ; [3]Merck Serono spa, Medical Affairs Department, Rome, Italy ; [4]National Cancer Institute- IRCCS Foundation G. Pascale, Medical Oncology-Department of Senology, Naples, Italy

Study question: Is luteinizing hormone (LH) able to protect ovarian reserve against cyclophosphamide (CPM)-induced damage in women?

Summary answer: LH appears able to significantly reduce primordial follicle (PMF) loss in ovarian strips treated *in vitro* with phosphoramide mustard (PM), the active metabolite of CPM.

What is known already: Cancer therapies cause a severe reduction of PMFs, often inducing premature ovarian insufficiency (POI) in female patients. For this reason, several compounds have been analyzed as adjuvant therapies to protect the ovarian reserve without interfering with cancer treatment on tumor cells. We recently demonstrated, *in vitro*, the protective effect of LH against ovotoxicity induced by cisplatin on the ovary of prepuberal mice. These results suggest a possibility for to use of LH prior or in concomitance with an anticancer treatment in order to preserve oocytes in human patients, therefore preventing the early onset of menopause and/or infertility.

Study design, size, duration: The ovarian tissues were collected from three patients (age-pathology : 25 yrs-Ewing's sarcoma, 15 yrs-medulloblastoma and 14 yrs-Hodgkin lymphoma) who have cryopreserved their ovarian tissue before receiving anticancer treatment. For each patient, eight ovarian strips were thawed and randomly assigned to the experimental conditions (two strips each): Control (CTRL), PM, PM+LH-200mIU and PM+LH-500mIU. LH was added 1 hour before the treatment with PM. Samples were analyzed after 24 and 48 hrs of treatment.

Participants/materials, setting, methods: Ovarian strips were cultured for 24-48 hours at 37°C and 5% CO_2, in α-MEM supplemented with 40% human serum with/out 10mM PM and/or 200mIU/ml LH or 500mIU/ml LH. The samples were processed for:

–Histology, for PMFs and primary follicles (PFs) analysis;

–Western blotting, for the expression of protein involved in DNA damage and apoptosis;

–Real-Time PCR, for the expression of genes involved in apoptosis and inflammation.

Main results and the role of chance: Histological analysis showed that in the untreated group, the follicle density varied from 450.45 to 921.38 PMFs/mm^3 and from 94.45 to 430.30 PFs/mm^3 between the three patients analysed. Therefore, relative follicular density (%) was performed to analyse statistical differences between the control and treated groups. Notably, whereas relative PMFs density (%) was significantly reduced in PM *vs* CTRL either after 24 and

48 hrs, this reduction was partially counteracted by both LH dosages tested (24 hrs: CTRL=74.47±5.12; PM=42.31±5.66; PM+LH-200mIU=70.77±3.72; PM+LH-500mIU=59.19±9.49 - 48 hrs: CTRL=68.15±1.75; PM=25.96±16.91; PM+LH-200mIU=42.62±11.79; PM+LH-500mIU=56.35±12.96). No significant differences were observed in relative PFs density (%). Real-Time PCR and Western blotting revealed no significant differences, between groups, in the expression of molecules involved in apoptosis (NOXA, PUMA, Bax, Bak and Bcl2), DNA damage (γH2AX), probably due to the already occurred loss of the follicles, and in the expression of IL6, a pro-inflammatory cytokine.
On the contrary, treatment with PM is able to significantly increase IL1β gene expression, another pro-inflammatory cytokine; interestingly, pre-treatment with LH, at both tested dosages, counteracted this increase (24 hrs treatment: CTRL=1.00±0.01; PM=2.03±0.04; PM+LH-200mIU=1.37±0.04; PM+LH-500mIU=1.50±0.03 and 48 hrs treatment: CTRL=1.00±0.12; PM=1.75±0.09; PM+LH-200mIU=1.30±0.10; PM+LH-500mIU=1.63±0.20).**Limitations, reasons for caution:** Main limitation of this preliminary study includes its small sample size (only ovarian cortex from three subjects were used) that needs to be improved. The present study did not elucidate the molecular mechanisms involved in LH ovoprotection against PM
Wider implications of the findings: These preliminary results showed that LH is able to preserve PMFs in human ovarian cortex exposed to PM *in vitro*. These findings encourage the possibility to use the hormone as a treatment to prevent the premature onset of menopause and/or infertility in women undergoing anticancer treatments.
Trial registration number: Not applicable

P-439 Production of ovine fertilizable oocytes using innovative in vitro (iv) technologies applied to preantral follicles growth.

C. Di Berardino1, G. Capacchietti[1], N. Bernabò[1], M. Crociati[2], M. Monaci[2], L. Sylla[2], B. Barboni[1]

[1]Faculty of Bioscience and Agro-Food and Environmental Technology- University of Teramo, Unit of Basic and Applied Biosciences, Teramo, Italy ;
[2]University of Perugia, Department of Veterinary Medicine, Perugia, Italy

Study question: Considering the early pool of gametes potentially able to generate offspring, what actions should be taken to optimize *iv*F thus increasing fertilizable oocytes availability?
Summary answer: The standardization of long-term gonadotropin stimulation is a key step to implement *iv*F in order to promote both reproductive targets, oogenesis and follicle steroidogenesis activation.
What is known already: Increasing in childhood cancer survivors points up an urgent need in fertility preservation (FP) strategies to overcome the negative impact of cancer treatments on reproductive cell cycle. Because of a concern exists on the possible re-introduction of malignant cells following ovarian transplantation, *in vitro* (*iv*) culture of early stage follicles still remains a potential alternative to obtain fertilizable oocytes that contribute to biological offspring. Since technologies advances are required to transfer *iv* Follicologenesis (*iv*F) from bench to bedside, monovulatory large mammal becomes ideal translational models to validate cultural conditions enhabling synergic follicles-oocyte *iv* development.
Study design, size, duration: The present research aimed to study the influence of 2 different gonadotropins (oFSH *vs* Pregnant Mare's Serum Gonadotropin/PMSG) on *iv*F cultures of single ovine preantral follicle (PA). The *iv*F outcomes obtained by using the specie-specific (oFSH) or the trans-species chorionic gonadotropin (PMSG) were analyzed after 14 days of culture by comparing follicle/oocyte growth, timing and percentage (%) of antrum differentiation as well as % of Methaphase II (MII) oocytes after *iv* Maturation (IVM).
Participants/materials, setting, methods: Preantral follicles, mechanically isolated from slaugterhoused prepubertal ovaries, were incubated as 3D single follicle culture and exposed to 25 ug oFSH or to different PMSG doses (from 0,4 to 40 IU/ml). In order to compare the degree of meiotic competence, MII oocytes obtained adopting an advanced IVM, consisting of cumulus oocytes complexes co-cultured with walls antral follicle-derived somatic cells, were analyzed.
Main results and the role of chance: The PMSG influence on *iv*F performances was strictly dose-dependent. More in detail, 4 IU was the concentration of PMSG able to enhance follicular diameter increment (Δ%: 63.7±28.9 *vs* 43.4±19.3 and 52.7±33.4, respectively for 4, 0.40, 40 IU; $p<0.05$). Conversely, any significant increase in antrum differentiation upon PMSG treatment (4, 0.4, 40 IU) was observed. The chorionic gonadotropin was more effective than oFSH in promoting *iv*F outcomes. Indeed, preantral follicles stimulated with 4 IU PMSG displayed a significantly greater growth than those exposed to oFSH (Δ%: 63.7±28.9 vs 52±31; p=0,038). Moreover, PMSG promoted a complete meiotic competence in the majority of collected oocytes (58.3% MII, 2.8% GVBD, 38.8% GV). Of note, the MII oocytes were all isolated by early antral follicles with Δ%>40 ($p<0.01$). On the contrary, only a very small fraction of the oocytes isolated from oFSH-treated follicles was able to resume meiosis and no one reached the MII stage (83,30% GV, 16,70% GVBD; $p<0.001$).
Limitations, reasons for caution: -
Wider implications of the findings: These proof-of-concept PMSG experiments establish a way for new protocols optimizing *iv*F and allow to identify a trans-species hormone to functionalize biomaterials. Both strategies might support follicle development in large animal models and humans with the hope of translating this technology for fertility preservation purposes.
Trial registration number: not applicable

P-440 Fertility preservation in adolescent males: Setting up a national service.

M. Horan[1], L. Hartigan[1], H. Groarke[1], L. Glover[1], M. Wingfield[1]

[1]Merrion Fertility Clinic- Dublin and The National Maternity Hospital Dublin, Obstetrics & Gynaecology, Dublin, Ireland

Study question: Does a multi-disciplinary approach to sperm cryopreservation in adolescent males enable quality care?
Summary answer: Within 18 months of inception, an efficient national care pathway has been established with successful cryopreservation and positive patient feedback.
What is known already: The incidence of both childhood and adolescent cancer is increasing throughout Europe. Around 200 children and young adolescents are diagnosed with cancer in the Republic of Ireland every year. Thanks to advances in medicine and surgery, survival rates in this group are now greater than 80%. As a result, there is increased emphasis on the long-term effects of cancer treatment. Studies from other units outside of Ireland have shown that semen cryopreservation was possible in most adolescent cancer cases regardless of age or disease diagnosis, however to date, no formal service for CAYA male sperm cryopreservation exists in Ireland.
Study design, size, duration: We developed a National CAYA Fertility Preservation Consortium with fertility specialists at our clinic and paediatric oncology specialists at the National Children's Hospital. The infrastructure and combined expertise through this collaborative consortium allowed us to provide a sperm cryopreservation service for adolescent boys, with the option of producing samples at either site. We performed a retrospective review of all adolescent male patients referred for consideration of sperm cryopreservation in a 16-month period.
Participants/materials, setting, methods: Patients were identified through the National Paediatric Haematology/Oncology Programme. Clinical and demographic data was recorded on patients. Disease diagnosis was provided by the primary oncologist or rheumatologist in the setting of benign disease. We evaluated patient demographics and semen parameters. Sperm parameters analyzed were volume, sperm concentration and motility. Number of failed samples, number of straws stored per patient, and number of visits required for each patient to produce a sufficient sample was also recorded.
Main results and the role of chance: This project addressed deficiencies and current barriers in the provision of fertility preservation services to children in Ireland.

Fifteen patients, aged between 12 and 17 years old, were referred to our service for consideration of sperm cryopreservation between August 2018 and December 2019. Of these, 93% (14/15) presented with malignant disease. One patient was referred for sperm cryopreservation prior to commencing gonadotoxic treatment for non-malignant disease. 93% of the male patients elected to come to our fertility clinic rather than provide their samples at the National Children's Hospital.

Of the 15 patients referred, 12 attempted sperm production. Eight of these patients (67%) had sperm successfully cryopreserved. The youngest patient in our study was 12 years old and successfully cryopreserved four straws. Four patients (36%) did not achieve sperm cryopreservation: 1 patient failed to collect

any sample, 2 patients had no sperm in the sample produced. Unfortunately 1 patient had very poor semen quality that was not suitable for freezing, this was following one cycle of chemotherapy. Five patients attended the clinic twice to attempt specimen production. Of the 8 patients who successfully had sperm cryopreserved, only 1 patient had semen analysis results entirely within WHO 'normal' limits.

Limitations, reasons for caution: The study cohort was limited in size. We currently have no long term data on the rate of straw utilisation in our cohort of patients, given that this is a very new service.

Wider implications of the findings: Prior to this study, the number of adolescent males availing of cryopreservation in Ireland was unknown. This study will enable collection of data and the establishment of a national database. Success thus far will improve awareness of this service among oncologists in Ireland.

Trial registration number: N/A

P-441 Sperm cryopreservation in adolescents with cancer.

F. Filippi[1], E. Somigliana[1,2], M. Reschini[1], C. Meazza[3], M. Podda[3], C. Guarneri[1], D. Giacchetta[4], M. Massimino[3], L. Restelli[1], M. Terenziani[3]

[1]IRCCS Fondazione Ca' Granda Policlinico di Milano, IVF Unit, Milano, Italy ; [2]Università degli Studi di Milano, Dept. Clinical sciences and Community Health, Milano, Italy ; [3]IRCCS Istituto Tumori di Milano, Pediatric Unit, Milano, Italy ; [4]Ospedale Santi Paolo e Carlo, Assisted Reproduction Techniques Unit, Milano, Italy

Study question: Is sperm banking underused in adolescents with cancer?

Summary answer: Semen cryopreservation prior to initiate gonadotoxic therapies is insufficiently used in adolescents with cancer.

What is known already: Sperm cryopreservation was the first validated technique for fertility preservation. It is simple, promptly available, cheap and safe. Even if only a minority of subjects who stored their semen will ever thawed them, there is a general consensus that sperm cryopreservation could be cost-beneficial and that it should be systematically offered to men with cancer prior to initiate gonadotoxic treatments. However, evidences on sperm cryopreservation in adolescents and young men are sparse. They generally show that sperm banking is feasible but also indirectly suggest that the proportion of those actually storing their semen may be hindered.

Study design, size, duration: Men undergoing semen cryopreservation at the Infertility Unit of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, Italy between January 1986 and December 2018 were retrospectively reviewed. Information were obtained from patients' charts.

Participants/materials, setting, methods: Inclusion criteria were as follows 1) Age 14-21 years, 2) diagnosis of malignancy. Subjects those performing surgical sperm collection were excluded. The analysis focussed on the following information: age at semen preservation, semen parameters at the time of preservation and oncological diagnosis.

Main results and the role of chance: Overall, 317 subjects satisfied our selection criteria. Cancer diagnoses were as follows: lymphomas (n=159, 50%), leukemia (n=21, 7%), testicular cancer (n=60, 19%) and others (n=77, 24%). The frequencies of referred cases according to age was not consistent ($p<0.001$) with the theoretical uniform distribution of referrals (ie with a similar number of cases per group of age): a clear sharp and constant increase with age emerged. This unbalanced distribution remained also when restricting the analysis to boys older than 16 years ($p<0.001$) or to those older than 18 years ($p=0.01$), suggesting that discrimination for age persisted also in older boys. The total number of cases referring before 1999, between 1999 and 2008 and between 2009 and 2018 were 88, 98 and 131, respectively. An improvement of the distribution of cases per age group emerged over time (Chi square for trend, $p=0.002$). However, within each historical period, distribution per age group remained inconsistent with the theoretical uniform distribution ($p<0.001$ for all). No statistically significant differences were identified in semen quality between the different age groups. The proportion of boys who could not freeze their specimen because of the absence of viable spermatozoa was also similar.

Limitations, reasons for caution: Number of boys who failed to make a collection was not recorded. Secondly, we cannot exclude that our results may reflect a local low sensitivity towards fertility preservation. Thirdly, we hypothesized a uniform distribution model for the referrals, assuming therefore that the frequency of cancer is constant over the years.

Wider implications of the findings: The frequency of adolescents who bank their semen prior to initiate gonadotoxic therapies is lower than it should be. Future research is needed to delineate the barriers and to develop interventions that could overcome them. Meanwhile, physicians engaged in oncology should dedicate more attention on fertility preservation in young boys.

Trial registration number: not applicable

P-442 Females' fertility preservation for cancer patients: lessons learned of ten years' experience in a French center

A. LE BRAS[1], V. Windal[1], V. Puy[1], L. Hesters[1], V. Gallot[2], B. Alexandra[2], M. Grynberg[2], C. Sonigo[2], N. Frydman[1]

[1]Hospital A. Béclère, Reproductive Biology Unit CECOS, Clamart Cedex, France ; [2]Hospital A. Béclère, Department of Reproductive Medicine and Fertility Preservation, Clamart Cedex, France

Study question: For female cancer patients who underwent fertility preservation, what are the outcomes of oocyte- or embryo-thawing after Controlled Ovarian Stimulation or *in vitro* maturation ?

Summary answer: Regardless of FP technique, live births were obtained for all of the emergency *in vitro* fertilization procedures in former cancer patients.

What is known already: Cryopreservation of embryos was considered the best FP method before the development of vitrification. Whereas vitrification has improved the success rate for cryopreserved oocytes in female cancer patients, no live births have ever been reported using in vitro matured oocytes.

Study design, size, duration: This was a single-center, retrospective study from January 2009 to December 2018 in France. We reviewed the biological and clinical outcomes of female patients treated for cancer who returned to use their oocytes or embryos that were cryopreserved using Controlled Ovarian Stimulation (COS) or In Vitro Maturation (IVM).

Participants/materials, setting, methods: After a cancer diagnosis, four possible cryopreservation procedures were used: either frozen oocytes or frozen embryos, after either COS or IVM treatment cycles. Among the 667 patients who underwent emergency FP before gonadotoxic cancer treatments, 40 returned to thaw their oocytes or embryos. We compared the clinical and laboratory data of freeze/thaw cycles for these four groups in these 40 patients.

Main results and the role of chance: Following emergency FP, 40 patients (6%) returned to attempt a pregnancy with a mean time lapse of 3.3 ± 1.5 years. Most were cured of breast cancer (n = 31). One hundred and thirty-four oocytes were used in 25 thawing cycles (7 after IVM-FP and 18 after COS-FP). Eighty-seven embryos were used in 24 thawing cycles (14 after COS-FP and 10 after IVM-FP). Eight pregnancies (3 from frozen oocytes, 5 from frozen embryos) resulted, three of them from fertilized, mature oocytes after IVM. All babies were delivered at term with normal perinatal characteristics. We report the first births in previous cancer patients using vitrification of *in vitro* matured oocytes for FP.

Limitations, reasons for caution: Some confounding factors such as smoking, alcohol consumption, and toxic environmental exposure at the time of cryopreservation are missing. Only a few patients have returned to attempt pregnancy after being cured from cancer. We have no information about natural pregnancies in patients who have had FP.

Wider implications of the findings: The present study is one of only a few published series conducted in patients treated for cancer comparing the outcomes of thaw cycles according to the FP procedure chosen at the time of diagnosis. We report the first pregnancy obtained from matured oocytes after IVM.

Trial registration number: NA

P-443 Effects of high concentrations of Resveratrol on human sperm cryopreservation

E. Porcu[1], L. Notarangelo[1], L. Cipriani[1], G. Damiano[1], P.M. Ciotti[1], N. Calza[1], S. Zuffa[1], M. Nadalini[2], E. Nardi[3], E. Borini[2], A. Borini[2]

[1]Infertility and IVF Unit, University of Bologna-Sant'Orsola Hospital, Bologna, Italy ; [2]9.baby, Family and Fertility Center, Bologna, Italy ; [3]University of Bologna, Department of Medical and Surgical Sciences, Bologna, Italy

Study question: Can high concentrations of resveratrol as antioxidants in sperm cryopreservation be useful for post thawing viability and morphology?
Summary answer: High concentrations of resveratrol do not improve vitality and post-thaw morphology in sperm cryopreservation.
What is known already: It was demonstrated that the cryopreservation of human semen produces reactive oxygen species (ROS), which cause important sperm damages such as lipid peroxidation of the cell membrane and DNA damage. Several studies have examined the role of in vitro supplementation of antioxidants, such as resveratrol, to protect sperm DNA from ROS oxidative damage and improve post-thawing sperm quality.
Study design, size, duration: The effects of Resveratrol will be examined based on the assessment of sperm motility and morphology. Prospective study: 100 waste seminal samples of patients undergoing a IVF cycle at the University of Bologna Infertility and IVF Center and 9.baby Center, Bologna from March to December 2019. Three groups were considered: Group A = post thawing control group; Group B = post thawing 30 µM/L Resveratrol; Group C = post thawing 50 µM/L Resveratrol.
Participants/materials, setting, methods: One hundred patients selected for normozoospermia and mild or moderate oligoastenoteratozoospermia. Patients age ranged from 23 to 53 years with a mean value of 38.18±6.00. Diagnosis of normozoospermia and mild or moderate oligoastenoteratozoospermia was made considering not only the cellularity of seminal fluid samples but also the total and progressive motility and morphology of spermatozoa. Sperm cryopreservation was performed using Test Yolk Buffer, Resveratrol and Sherman freezing Protocol.
Main results and the role of chance: Semen standard analysis showed that in the pre-freezing group the parameters related to total motility, progressive motility and morphology were 48,70 ± 8,63, 24,45 ± 14,70 and 19,73 ± 9,32 respectively. In the control group, total motility, progressive motility and morphology were respectively 26,30 ± 7,09, 12,30 ± 8,39 and 13,60 ± 6,59. In the 30 µM/L Resveratrol group the total motility, progressive motility and morphology were respectively 22,77 ± 6,72, 9,47 ± 6,48 and 13,05 ± 6,41. In the 50 µM/L Resveratrol group total motility, progressive motility and morphology were respectively 19,05 ± 6,54, 6,87 ± 5,71 and 11,83 ± 6,09. Thawing results showed that, 30 µM/L and 50 µM/L of resveratrol produce pejorative effects on motility and morphology ($P < 0.0001$) of sperm after thawing, compared to the control group.
Limitations, reasons for caution: The scientific community opinion is still controversial about the use, in vivo and in vitro, of antioxidants in IVF cycles.
Wider implications of the findings: In IVF cycles, the occurrence of oxidative events during in vitro culture and in cryopreservation processes is well demonstrated; the use of antioxidants during these phases must be further investigated.
Trial registration number: None

P-444 Mitochondrial activity in prepubertal and adult ovaries

R. Masciangelo[1], M.C. Chiti[1], A. Camboni[1], C.A. Amorim[1], J. Donnez[2], M.M. Dolmans[3]

[1]Université Catholique de Louvain, Pôle de Recherche en Gynécologie- Institut de Recherche Expérimentale et Clinique, Bruxelles, Belgium ;
[2]Société de Recherche pour l'Infertilité, Avenue Grandchamp 143, Bruxelles, Belgium ;
[3]Cliniques Universitaires Dt. Luc, Départment de Gynécologie, Bruxelles, Belgium

Study question: Are mitochondrial content and activity in prepubertal ovarian follicles different from adult ovarian follicles?
Summary answer: Differences in the number, activity and morphology of mitochondria of prepubertal patients were evidenced.
What is known already: Mitochondria generate most of the energy of cells through oxidative phosphorylation, possible thanks to generation of mitochondrial membrane potential. Reduced efficiency of oxidative phosphorylation and energy production in oocytes is related to poor embryo development. Moreover, it is known that mitochondria play a central role in ovarian aging via their major contribution to cell survival and apoptosis and that maternal age is associated with increased oxidative stress in oocytes, resulting in mitochondrial dysfunction. The role of mitochondria in determining oocytes' developmental capacity is increasingly evident, nevertheless, literature on mitochondrial function in prepubertal follicles at early stages of growth is scarce.
Study design, size, duration: Ovarian tissue was collected from 7 prepubertal girls (age 1-10 years) and 6 adult women (age 20-35 years). Primordial and primary follicles were isolated from frozen-thawed prepubertal and adult ovarian tissue. Frozen-thawed ovarian tissue from the same patients was also evaluated by immunohistochemistry and transmission electron microscopy.
Participants/materials, setting, methods: Mitochondrial activity in isolated follicles was explored by MitoTracker CM-H2XRos, which is a mitochondria-specific fluorescent and cell-permeant probe, selectively sequestered only in mitochondria with active $\Delta\Psi m$, depending on their oxidative activity. Mitochondrial content was investigated in prepubertal and adult ovarian tissue by TOMM20 immunostaining, a peptide receptor located on the surface of the outer mitochondrial membrane. Transmission electron microscopy was performed to evaluate mitochondrial morphology.
Main results and the role of chance: Prepubertal follicles showed higher fluorescence intensity by MitoTracker ($p=0.03$), compared to adult follicles. This result suggests that mitochondrial activity is enhanced in prepubertal patients compared to adult patients. Quantification of TOMM20 immunostaining, based on the intensity and extent of the signal, revealed significantly stronger TOMM20 staining in prepubertal follicles than adult follicles ($p=0.01$), indicating the presence of more mitochondria in prepubertal follicles. Last, TEM allowed to identify ultrastructural differences in the morphology of mitochondria: in prepubertal follicles, mitochondria were heterogeneously dispersed throughout the cytoplasm in small clusters and were mostly elongated in shape, rather than round as in adult mitochondria. Moreover, cristae in prepubertal mitochondria were mostly longitudinal rather than archiform and, in some cases, shaped in the form of a fingerprint.
Limitations, reasons for caution: The present study evaluates follicle maturation and competence in prepubertal patients, by assessing mitochondria function and activity. Nevertheless, these parameters only provide indirect information and additional cellular organelles should be studied to further corroborate these results.
Wider implications of the findings: With this study, we provided for the first time information on the function, content and morphology of mitochondria from prepubertal patients. We revealed that in prepubertal follicles mitochondria are more abundant and that mitochondrial activity is enhanced, suggesting that intrisic maturation changes will occur in ovarian follicles with aging.
Trial registration number: /

P-445 Elective fertility preservation: do women come back as much as they freeze?

M. Miguens[1], A.M. Quinteiro Retamar[2], A. Coscia[2], D. Acosta[2], G. Vega Balbuena[1], N. Allende[2], S. Papier[2]

[1]cegyr, fertility, buenos aires, Argentina ;
[2]cegyr, clinical, buenos aires, Argentina

Study question: What is the return rate for counseling in patients who had chosen to do elective fertility preservation and the proportion of women who used their stored eggs?
Summary answer: A quarter of patients who preserved their fertility return around two years after for counseling and only 12,58% of the total performed an IVF cycle.
What is known already: Increasing numbers of women are seeking elective oocyte cryopreservation (EOC). This is due to the improving success rates of the oocyte vitrification technique over the years and also social freezing is promoted to single women as a way of preserving their fertility potential. Recent studies have found usage rates are less than ten percent. Studies have proven that vitrified oocytes can be fertilized, implant and achieve normal pregnancies after a short period but the chance of having desired outcomes in long term cannot be predicted by physicians based on real-life experience.
Study design, size, duration: A cross-sectional retrospective study of 469 women who did an EOC between 2009 and 2018 in a single-center at Buenos Aires, Argentina.
Participants/materials, setting, methods: Women who did an EOC between 2009 and 2018 were included. The primary outcomes were the return rate to the clinic for counseling and the proportion of women who used their storage eggs. Other analyzed variables were mean age at the moment of the freezing, mean number of oocytes vitrified per patient, the average time it took

patients to return and ongoing pregnancy rate in the patients who used their oocytes. All analyses are descriptive.
Main results and the role of chance: The total of cryopreserved oocytes over the years was 3376 being the average 7,2 per patient (from 1 to 32). The mean age at the moment of the EOC was 37,17 years (19 to 45). The 24,95% (n: 117) of the total of patients returned for counseling with a mean age of 39,57. The average time it took women to return was 2 years. From this group, 27,35% (n:32) have decided to perform a second oocyte retrieval cycle (main reason was a low number of eggs vitrified in the cycle before -median: 4-) and around a half-used their stored eggs for an IVF treatment (50,43% n:59) which is a 12,58% of the total of patients included. The thawed oocyte viability rate was 72%, the fertilization rate was 66% and the ongoing pregnancy rate was 32.2% (n:19).
Limitations, reasons for caution: The main limitation of this study was its retrospective design based on data from a single center which may be subject of bias.
Wider implications of the findings: The cycles of oocyte cryopreservation, in our clinic, have increased at least 800% since 2009. Even though, our rate is a little above compared with the literature, is still a low number. Prospective cost-effectiveness and long-term success analysis are needed to determine if it's worth it or not.
Trial registration number: Not applicable

P-446 Ascorbic acid protects against ovarian dysfunction induced by mono (2-ethylhexyl) phthalate in fetal mouse ovaries in vitro via reducing the oxidative stress

C. Sui[1], C. Liu[1]

[1]Tongji Hospital- Tongji Medical College- Huazhong University of Science and Technology, Reproductive Medicine Center, Wuhan, China

Study question: Whether ascorbic acid protects fetal ovaries from the injury effect induced by mono (2-ethylhexyl) phthalate (MEHP) and what is the mechanism.
Summary answer: Supplement of ascorbic acid can rescue the injury effect induced by MEHP in fetal mouse ovaries in vitro via reducing the oxidative stress
What is known already: Di-(2-ethylhexyl) phthalate is considered to be toxicant to female reproduction, potentially by its active metabolite MEHP. Toxicological studies affirmed oxidative stress is one possible pathway for MEHP-adjuvant effect. Ascorbic acid is known to be an antioxidant and a powerful reducing agent and plays an important role in attenuating oxidative damage to the reproductive system.
Study design, size, duration: Fetal mouse ovaries exposed to different concentration of MEHP (0 M, 10^{-4} M, 10^{-5} M, 10^{-6} M) were treated with or without ascorbic acid (100nM) in vitro for 6 days. Then the ovarian follicle constitution and the oxidative stress parameters of ovaries in each treatment were detected respectively to evaluate the influence of MEHP and ascorbic acid.
Participants/materials, setting, methods: Ovaries from post-natal day 4 Kunming mice were recruited and assigned to different groups randomly. After treated with MEHP alone or a combination of MEHP and ascorbic acid for 6 days, ovaries were collected to histologically qualify the follicle numbers, and the ovarian malonaldehyde (MDA) levels were tested. Besides, ovarian mRNA related to Redox (Sod-1, GSS, CAT) were examined using quantitative reverse transcription polymerase chain reaction.
Main results and the role of chance: MEHP accelerated primordial follicle activation by increasing the proportion of primary and secondary follicles in ovaries at the concentration of 10^{-4} M. The expression of SOD was increased significantly in ovaries treated with 10^{-4} M MEHP ($p<0.05$). Moreover, MEHP increased the MDA level in the ovaries at 10^{-4} M ($p<0.01$) and 10^{-5} M ($p<0.05$). When ovaries exposed to 10^{-4} M MEHP, supplement of ascorbic acid rescued the follicular constitution, and normalized the MDA level. Ascorbic acid also reversed the increasing expression of SOD and GSS in the ovaries, which was caused by MEHP.
Limitations, reasons for caution: This study only demonstrated the protective effect of ascorbic acid to ovaries in vitro. Whether ascorbic acid functioned as a powerful agent to rescue the MEHP-induced ovarian injury will need in vivo studies to explore.
Wider implications of the findings: Ascorbic acid ameliorated the disruption of folliculogenesis induced by MEHP via its antioxidant capacity. Supplement of ascorbic acid might be a potential therapy for MEHP-induced ovarian injury.
Trial registration number: not applicable

P-447 Fertilisation outcomes after cryopreservation of human immature and in vitro matured oocytes

M. Petrushko[1,2], V. Piniaiev[3], T. Yurchuk[2]

[1]ART Clinic of Reproductive Medicine, Embryology, Kharkiv, Ukraine ;
[2]Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine, Cryobiology of reproductive system, Kharkiv, Ukraine ;
[3]ART Clinic of Reproductive Medicine, Gynecology, Kharkiv, Ukraine

Study question: What are maturation and fertilization outcomes of GV oocytes in vitro matured prior and after vitrification?
Summary answer: Oocytes in vitro matured prior vitrification have higher maturation, fertilization and blastocyst formation rate than oocyte matured after vitrification.
What is known already: Emergency of fertility preservation for patients who are in danger of losing their ovarian function or donation programs apply oocyte preservation for further usage in assisted reproductive technologies in spite of being at different maturation stage (germinal vesicle stage (GV), MI, MII). There is lack of data concerning oocyte cryopreservation efficiency and timing of in vitro maturation regards to cryopreservation procedure.
Study design, size, duration: GV oocytes (n=108) were cryopreserved prior and after *in vitro* maturation oocytes and their survival, fertilisation and blastocyst formation rates were analyzed. All manipulations have been carried out between January 2017 and November 2019.
Participants/materials, setting, methods: This single-center cohort study included 31 patients diagnosed with polycystic ovary syndrome (PCOS) and poor responders (mean age of women was 34.3±4.2 years). GV oocytes were randomly selected for *in vitro* matured (IVM) prior (group 1) and after vitrification (group 2). After thawing MII oocytes were fertilized by ICSI and cultured up to day 5. All manipulations with gametes were performed according with written consent of patients.
Main results and the role of chance: It has been shown maturation rate was 73.2 *vs* 52.0% in group 1 and 2 respectively. Overall no differences were found for survival rate of MII oocyte matured prior cryopreservation and cryopreserved GV oocyte (97.6 % vs 96.2 %; $P>0.05$). Fertilisation and blastocyst formation rate were higher in group 1 (77.5 % and 38.7%) than in group 2 (38.5% and 20.0%). These differences are significant between the groups ($P<0.05$).
Limitations, reasons for caution: Limited number of GV oocytes in the study group. Further studies are recommended and should include also group advanced age patients.
Wider implications of the findings: These results revealed that using immature oocytes at GV stage and long-term preservation can increase the number of embryos available for transfer into the uterus of patient with PCOS and poor responders.
Trial registration number: not applicable

P-448 Fertility preservation in adolescent women: should we expect the same response?

A. Santolaria Baig[1], R. Quintana Berto[1], N. García Camuñas[1], J. Subirá Nadal[1,2], P. Polo Sánchez[1], J.M. Rubio Rubio[1]

[1]La Fe University Hospital, Women's Health Area, Valencia, Spain ;
[2]IVI-RMA Castellón-Valencia, Reproductive Endocrinology, Castellón, Spain

Study question: Are oocyte cryopreservation (OC) cycles in adolescent women (<20 years old) comparable to OC cycles in ages we expect the best response (20-25 years)?
Summary answer: OC cycles in adolescent women have similar results compared with ages we expected the best response.Fertility preservation(FP) is a feasible option in this age group.
What is known already: Women from 20 to 25 years old are expected to have the best response to OC cycles for FP, based on the age-related distribution of AMH. Few studies are published in adolescents (<20 years), even though FP in this group of patients is increasing nowadays. Some authors hypothesized that adolescent have differences in their stimulation cycles regarding the oocyte

maturity or dosing requirements of gonadotropins.The last publications obtained similar results in stimulation in adolescents and in women of other age groups, but with a higher likelihood of cancellation.

Study design, size, duration: This is a retrospective study performed from January 2013 to January 2020 at a tertiary university hospital. Our research was focused in OC cycles of adolescent women (between 13 and 20 years of age) and the group we expected the best response according to AMH levels (20-25 years). Data were retrieved from patients electronic records.

Participants/materials, setting, methods: We collected data from epidemiology, stimulation and oocyte retrieval from 49 cases in adolescent women and 104 cases in the 20-25 years group. Numerical variables were summarized by the median and standard deviation and a comparation of both groups was performed with T Student test.

Main results and the role of chance: The main indication in adolescents for FP was Hodgkin lymphoma(36,73%) followed by gynecological pathology(24,48%). In the 20-25 years group the gynecological pathology was the main indication(26,92%), followed by Hodgkin lymphoma(22,11%) and breast cancer (9,6%).

Both groups were comparable in terms of epidemiological variables about ovarian reserve. In all ages, the most common stimulation protocol was short antagonist protocol.

Results from total days of stimulation, total number of follicles, follicles >16 mm and serum estradiol on the trigger day and FORT(Follicular Output Rate) index were similar in both groups. Regarding the gonadotropin dosing, adolescent used a mean FSHr dosed of 2219,46 IU(±909,88) compared with 2520,50 IU(±934,79) in women 20-25 years(p=0,07). However the initial dosing was higher in the 20-25 years group: 222,90 IU(±53,66) vs 246,39 IU(±52,53) (p<0,05). No significant differences were found between the groups for oocytes retrieval or the maturity rate. Adolescents retrieved a mean of 13,69(±10) oocytes with a maturity rate of 67,76%(±28,32) compared with 11,53(±8,15) oocytes and 75,42%(±23,45) in women 20-25 years. The Follicle to Oocyte Index (FOI) was also comparable in both groups.

There were more cancellations in the adolescent group:20,4 % of cycles in adolescents vs 6,7% in women 20-25 years(p=0,025).

Limitations, reasons for caution: The data from this study were retrospective and from a single center.

Wider implications of the findings: This study shows that OC cycles in adolescents have similar results to 20-25 years women, the age expected to have a better response.It is plausible to include adolescents in FP programs due to their good response. This is very reassuring because these patients are less likely to have completed childbearing.

Trial registration number: Not applicable

P-449 Developmental quality post-insemination of vitrified versus fresh oocytes from patients with low ovarian reserve

F. Bonald[1], M. Montagut[1], M. Clouet[2], V. Guitard[1], A. Chiron[1], D. Nogueira[3]

[1]Labosud Garonne, Reproductive Biology Laboratory, Toulouse, France ;
[2]Clinique Croix du Sud, Gynecology, Toulouse, France ;
[3]INOVIE Fertilité - Labosud Garonne, Reproductive Biology Laboratory - Clinique Croix du SUD, Toulouse, France

Study question: Does oocyte developmental quality is affected after vitrification in women with low ovarian reserve?

Summary answer: Embryonic developmental quality seems affected after vitrification compared to freshly collected oocytes from the same patient

What is known already: Oocyte vitrification in low ovarian reserve patients can be used for fertility preservation, to accumulate oocytes for optimizing the use of frozen-thawed-testicular and donor sperm, to augment the number of embryos in PGT cycles. Embryos from vitrified oocytes have similar implantation potential than freshly collected oocytes from infertile patients but the number of vitrified oocytes has an affect on delivery rates. Data on donors showed that in vitro development of embryos derived from vitrified-warmed donor oocytes is altered compared to their fresh sibling counterparts. Data on oocyte developmental quality after oocytes vitrification in patients with low ovarian reserve is lacking.

Study design, size, duration: A retrospective analysis performed between January 2016 and December 2018 including low ovarian reserve patients (AMH < 1.1 ng/ml by The Bologna criteria) (n=206). These patients underwent 1.6 ±0.9 previous ovarian stimulation cycles for oocyte vitrification, followed by a fresh oocyte retrieval where all oocytes previously vitrified were warmed for ICSI, as a strategy to increase the potential number of embryos available.

Participants/materials, setting, methods: Oocyte vitrification and warming were performed with the Cryotop method (Kitazato, Dibimed). Analysis on pre- and post-embryonic developmental quality and perinatal evaluation was performed after ICSI on 580 freshly collected oocytes (FO) and on 614 vitrified-warmed oocytes (VO) and fresh embryo transfer.

Main results and the role of chance: Patients had 36.4 ± 3.7-year-old, AMH of 0.7 ± 0.4 and FSH ± 10.3 4.1. The mean number of 2.8 ± 2.1 FO and 3.0 ± 2.2 VO were used for ICSI on the same day per patient. Although fertilisation rates were similar between FO and VO (64% vs 62%), embryonic developmental quality was affected on day 3 ($p < 0.01$) and day 5 ($p < 0.05$) of development being lower in vitrified oocytes compared to their fresh counterparts, which resulted in less embryos chosen for transfer in VO. A total of 161 patients underwent embryo transfer: 81 had 1.3 ± 0.5 embryos transferred from FO, while 49 received 1.3 ± 0.4 embryos from VO. Thirty patients received mix transfer (one FO and one VO). Implantation rates were 22% and 28% for FO and VO (NS), respectively. All embryos derived from vitrified oocytes that implanted progressed to delivery (n=14), while 3/17 (18%) and 3/8 (38%) embryos from freshly collected oocytes and mixed transfer miscarried. Perinatal outcomes from this preliminary evaluation showed no difference in gestational age and body weight of children between freshly collected (38.67 weeks and 3092 kg) and vitrified oocytes (39.07 weeks and 3298 kg) (NS).

Limitations, reasons for caution: Retrospective design of the study and limited sample size concerning the data on delivery rates.

Wider implications of the findings: In view of the lower in vitro developmental potential of embryos derived from vitrified oocytes in low ovarian reserve patients, accumulation of oocytes in patients undergoing ICSI cycles should be further evaluated. However, the preliminary data on perinatal outcomes seems to reassure the safety of this technology once implantation occurs.

Trial registration number: not applicable

P-450 Low value of AMH did not affect oocyte quality and pregnancy outcome in patients with severe endometriosis

A. Pacchiarotti[1]

[1]San Filippo Neri Hospital, IVF Unit, ROMA, Italy

Study question: The aim of this pilot study was to determine that low anti-müllerian hormone (AMH) serum levels due to severe endometriosis *did not affect IVF* clinical outcomes in young patients (<37)

Summary answer: young patients with low AMH level due to severe endometriosis displayed a diminished oocyte yield but not a reduction in embryo quality and pregnancy outcomes.

What is known already: diminished ovarian reserve is associated to reduced fertility and poor response to ovarian stimulation during *in vitro* fertilization (IVF) treatments

Study design, size, duration: A total of 50 IVF cycles of patients younger than 37 with severe endometriosis were retrospectively analyzed in a single center between November 2016 and July 2018 and compared to a control group of 84 patients with no story of endometriosis and normal AMH value. AMH value has been evaluated within three months before the stimulation. number and maturation of retrieved oocytes, embryo quality, and pregnancy outcomes were compared using Student's t-test and Fisher's test.

Participants/materials, setting, methods: A total of 50 IVF cycles of patients younger than 37 with severe endometriosis were retrospectively analyzed in a single center between November 2016 and July 2018 and compared to a control group of 84 patients with no story of endometriosis and normal AMH value. AMH value has been evaluated within three months before the stimulation. number and maturation of retrieved oocytes, embryo quality, and pregnancy outcomes were compared using Student's t-test and Fisher's test.

Main results and the role of chance: The number of oocytes retrieved per cycle and the percentage of mature oocytes (MII) were significantly (P<0.001) lower in IVF patients with severe endometriosis AMH value ≤ 1.1 ng/ml (Group A; 3.8±2.6 retrieved oocytes, 70% MII) compared to patients without endometriosis and AMH levels > 1.1 ng/ml (Group B; 6.9±4.6 retrieved oocytes, 83%

MII). On the other hand, embryo morphology, implantation rate (31% vs 33%; *P*=0.833) and pregnancy rate (50% vs 49%; *P*=1) were comparable in the two groups.
Limitations, reasons for caution: none
Wider implications of the findings: young patients with an impairment of the ovarian reserve due to severe endometriosis displayed a diminished oocyte yield but not a reduction in embryo quality and pregnancy outcomes. These results suggest that serum AMH levels should not be adopted as a criterion for discouraging those patients from undergoing IVF treatments.
Trial registration number: not applicable

POSTER VIEWING SESSION
NURSING AND MIDWIFERY

P-451 I trust the healthcare professional most: Exploring fertility knowledge among healthcare professional and lay population groups

B. Grace[1], J. Shawe[2], J. Sarah[3], S. Judith[1]

[1]University College London, Institute for Women's Health, London, United Kingdom ; [2]The University of Plymouth, Institute of Health and Community, Plymouth, United Kingdom ; [3]SPD GmbH, Clinical Research, Bedford, United Kingdom

Study question: What is the level of fertility knowledge amongst healthcare professionals compared to lay men and women in the UK?
Summary answer: Healthcare professionals in this study did not appear to have better knowledge than the lay population in this study.
What is known already: As the average age of first time parents continues to rise, health policies have highlighted the importance of optimising reproductive health through better knowledge and awareness. This study therefore aimed to assess current patterns of fertility knowledge, attitudes and practices; and identify improvement opportunities. In this study, fertility knowledge of lay men and women and HCPs was assessed using the same test instrument, which provided a new and unique perspective compared to previous studies.
Study design, size, duration: Mixed method research was conducted using a UK wide cross-sectional survey and semi-structed interviews. Results were obtained from 1,082 survey respondents which included 347 HCPs, 332 men, and 413 women, 115 of whom were trying to conceive. There was variation in age, level of education, ethnicity and training. Interviewees were purposively sampled to include men and women from the reproductive age range (18-45 years) and of varying ethnic and educational backgrounds.
Participants/materials, setting, methods: Survey participants were recruited nationwide via online newspaper and social media adverts. Of those who agreed to a follow-up interview, 35 were included this study. Healthcare professionals (HCPs) were recruited from professional bodies such as RCN, RCGP, RCP, doctors.org.uk. Interview data were transcribed and analysed using the framework method. Interviews lasted an hour on average. Favourable ethical opinion was given by University College London Research Ethics Committee.
Main results and the role of chance: Survey questions covered the following areas: demographics, basic definitions, male and female reproductive biology, age-related fertility decline and conception. The proportion of HCPs correctly answering the survey knowledge questions was 47.0%(95% CI= 41.6%,52.4%) compared to 47.3% for women(95% CI= 41.0%,52.2%); 50.9%(95% CI= 40.1%, 61.0%) for Women trying to conceive sub group; and 36.3% (95% CI= 30.8%,41.6%) for men. Chi-squared test confirmed statistically significant difference amongst groups. 98.8% of HCPs stated that they provide fertility advice in their role.

Although the internet and school education remained popular sources, HCPs were ranked as the most trusted source for seeking fertility and reproductive health information, however, they did not appear to have better knowledge than the lay population in this study. From interviews, there were inconsistencies among HCPs regarding where responsibility lies for providing the right information and support to patients on fertility awareness. Recommendations were made for additional training on the topic part of general HCP training.

"There is a lack of information. We do touch upon it but I think that with the current climate it's such a significant part of people's lives. I don't think there's enough emphasis on training clinical staff when it comes to fertility…" HCP3, Nurse.
Limitations, reasons for caution: One of the main methodological limitations of this study is the self-selection process, which has implications for generalisability. The results necessarily reflect the views of those who were willing to participate. For the lay population, the online recruitment method could result in potential bias towards respondents of higher socioeconomic status.
Wider implications of the findings: School education remains a consistent source of information but does not adequately cover fertility education. In addition to websites based on robust scientific evidence and centres specialising in sexual education; as an important source, there remains an important need for additional training for primary HCPs on reproductive health.
Trial registration number: N/A

P-452 The Gestational Diabetes Mellitus (GDM) and Risk Factors After Assisted Reproductive Techniques (ART)

N.N. Aypar Akbağ[1], M. Aluş Tokat[2], S. Fata[3], E. Ünsal Avdal[4], R.E. Okyay[3], Ö.E. Doğan[5]

[1]KaramanoğIu MehmetbeyUniversity, Gynecology Nursing, Karaman, Turkey ; [2]Work adres, Dokuz Eylul University Nursing Faculty, Izmir, Turkey ; [3]Dokuz Eylul University, Gynecology Nursing, Izmir, Turkey ; [4]Katip Çelebi University, Internal Medicine Nursing, Izmir, Turkey ; [5]Dokuz Eylul University, Gynecology Medicine, Izmir, Turkey

Study question: Do assisted reproductive techniques play a pivotal role in gestational diabetes mellitus (GDM) incidence and risk factors for GDM in pregnancies after ART?
Summary answer: The hormones stimulated in ART may cause lipid, carbohydrate metabolism disturbances. Obesity, age, polycystic ovary syndrome in addition to ART may increase incidence of GDM.
What is known already: External stimulation of hormones in assisted reproductive therapies is known to cause disharmony in lipid, carbohydrate and protein metabolism. However, it is stated in the literature that the increase in the incidence of GDM may be caused by some confounding factors such as age, obesity, polycystic ovary syndrome (PCOS) and multiple pregnancies developing after ART.
Study design, size, duration: The retrospective cohort study was performed between December 2018 and February 2019 by using the file information of women who had been treated at Dokuz Eylul University InVitroFertilization (IVF) Center and gave birth between 2006-2018. One hundred eigth women were reached.
Participants/materials, setting, methods: Women who treated at the IVF center between 2006-2018 were included. Giving birth after ART was inclusion criteria; Polycystic Ovarian Syndrome (PCOS), age 40 and over, Body Mass Index (BMI) 30 and over, glucose intolerance, previous GDM history and corticosteroid treatment were the exclusion criteria. The women were called from center and data was collected via telephone. A total 621 women registered in center, 108 women were reached.
Main results and the role of chance: The GDM was reported by 16.7% of women participating in the study. The results was interpreted as high incidence according to general incidence of GDM reported by American Diabetes Association's incidence of GDM (1-14%). Ovulation induction drugs used by all women were rFSH (recombinant Follicle Stimulating Hormone) (70.4%), Human Menopausal Hormone (HMG) (16.7%), FSH and HMG (13.0%). The mean drug starting dose was 257.98±110.95 and the total dose was 2797.47±1457.20 units. They used drugs for an average of 10.60±1.92 days. Progesterone use rate after transfer was 65.7%. Using rFSH, HMG, together rFSH and HMG (KW=1.162, p=0.281), drug starting dose (U=668.000, p=0.236), the total dose of drug (U=722.500, p=0.470), the duration of drug use (U=767.000, p=0.718), using or not using of progesterone after transfer (KW=0.204, p=0.652) did not increase the incidence of GDM.
Limitations, reasons for caution: Since the data were obtained from the sample treated in a wide period of time, problems related to the recall factor were experienced. Also, some women could not be reached from their registered numbers in the centers.

Wider implications of the findings: This study suggests that women who will receive ART should be screened for risk of GDM from the begining of pregnancy.The incidance can be higher in this group compared with natural pregnancy. Studies with larger sample should be performed for specific determimining the risk factors that can affect this process.
Trial registration number: not applicable

POSTER VIEWING SESSION
PSYCHOLOGY AND COUNSELLING

P-453 Eliciting couples' preferences for fresh versus elective frozen embryo transfer in IVF: a discrete choice experiment

B. Abdulrahim[1], G. Scotland[2], S. Bhattacharya[3], A. Maheshwari[4]

[1]Aberdeen Fertility Centre, University of Aberdeen, Aberdeen, United Kingdom ; [2]Institute of Applied Health Science, University of Aberdeen, Aberdeen, United Kingdom ; [3]School of Medicine- Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, United Kingdom ; [4]Aberdeen Fertility Centre, NHS Grampian, Aberdeen, United Kingdom

Study question: What are the factors influencing couples' preferences for fresh versus elective frozen embryo transfer (ET) in in-vitro fertilisation (IVF)?
Summary answer: Accepting that elective frozen ET can delay treatment but reduces the risk of OHSS, couples' choices are driven by expected pregnancy and birth outcomes.
What is known already: Elective frozen embryo transfer results in livebirth rates which are on par with, or better than those following fresh embryo transfer. In terms of safety, elective embryo transfer reduces the risk of ovarian hyperstimulation (OHSS) and small for gestational age babies but increases the risk of pre-eclampsia and large for gestational age compared to fresh embryo transfer. Given the prevailing uncertainty around a universal policy of elective frozen embryo transfer for all couples undergoing IVF, user preferences are critical in informing clinical decision making.
Study design, size, duration: Discrete choice experiments (DCEs) are a preference elicitation technique for assessing the value that individuals derive from different aspects of a treatment or service. We asked both partners of 104 IVF naïve couples attending a tertiary referral centre to independently complete a questionnaire with nine hypothetical choices illustrating potential advantages and disadvantages of fresh versus frozen embryo transfers in terms of four attributes (live birth rate, miscarriage rate, neonatal complication rate and cost of treatment).
Participants/materials, setting, methods: Participants were informed that frozen ET would delay the transfer of embryos by 6-8 weeks but reduce the risk of OHSS. An opt-out (no IVF treatment) was included for each choice task. Logistic regression was used to analyse the choice response data and estimate preference weights for fresh and frozen ET and each treatment attribute. Willingness to pay (WTP) was calculated as the marginal rate of substitution between the cost attribute and each other attribute.
Main results and the role of chance: A total of 104 couples completed 208 questionnaires. Couples preferred both fresh and elective frozen embryo transfer (Odds ratio 27.65 and 27.7 respectively) compared to no IVF treatment with no strong preference for fresh over frozen.

Couples strongly preferred any IVF treatment options that increased the livebirth rate by 5% (OR 1.28; 95% CI; 0.070 - 0.424), reduced the miscarriage rate by 18% (OR 0.54; 95% CI; 0.788 - 0.454) and diminished neonatal complication rates by 10% (OR 0.59; 95% CI; 0.697 - 0.357).

Respondents were willing to pay an additional £2452 (95% CI; 596 - 4,308) and £7,168 (95% CI 5,053 – 9,283) for a 5% and 15% increase in the live birth rate respectively. They required compensation of £5,230 (95% CI; 3,320 -7,141) and £13,245 (95% CI; 10,110 – 16,380) for treatments that increased the neonatal complication rate by 10% and 25% respectively. Couples valued a 10% diminution in the risk of neonatal complications and a 10% increase in the livebirth rate equally.

Older women put a higher monetary value on increases in the livebirth rate than younger women. Partners tended to place a greater value on reductions in treatment associated risks than those intending to get pregnant.
Limitations, reasons for caution: DCEs can elicit intentions which may not reflect actual behaviour. The external validity of this study is limited by the fact that it was conducted in a single centre with generous public funding for IVF.
Wider implications of the findings: Couples undergoing IVF may be willing to forgo higher livebirth rates for reduced maternal and perinatal risks. Along with evidence from randomised trials, these preferences should be used to inform policy as well as individualised decision making in IVF.
Trial registration number: Not applicable

P-454 Pre-implantation genetic testing for aneuploidy; motivations, concerns and perceptions in a UK population

B. Jones[1], T. Bracewell-Milnes[1], M. Spearman[2], M. Jalmbrant[3], J. Green[4], O. Rabi[4], S. Saso[1], P. Serhal[4], J. Ben-Nagi[4]

[1]Imperial College London, Department of Surgery and Cancer, London, United Kingdom ; [2]University College London, Medicine, London, United Kingdom ; [3]Imperial College NHS Foundation Trust, Clinical psychology, London, United Kingdom ; [4]The Centre for Reproductive & Genetic Health, Fertility, London, United Kingdom

Study question: What are women's motivations and concerns in using pre-implantation genetic testing for aneuploidy (PGT-A), and what are their perceptions after undergoing it as part of in vitro fertilisation (IVF) treatment?
Summary answer: Most women use PGT-A to optimise the chance of successful embryo transfers, are satisfied after treatment, and would use it in future IVF cycles
What is known already: PGT-A facilitates embryo selection by allowing the opportunity to prioritise chromosomally normal embryos for transfer to the uterus. The transfer of embryos adjudged to be euploid using modern PGT-A methods is associated with higher implantation, ongoing pregnancy and delivery rates with reduced pregnancy loss compared to unscreened embryos, thus mitigating the negative clinical impact maternal age has upon reproductive outcomes. Despite the upward trend in women utilising PGT-A, there is little data about the women's motivations and concerns toward using the technology, and perceptions having gone through the process.
Study design, size, duration: This was a cross sectional survey which included 161 women who used PGT-A in a single fertility clinic in the UK between 2014-2018.
Participants/materials, setting, methods: An online questionnaire was created to assess motivations and concerns toward PGT-A as well as outcomes and perceptions following IVF using PGT-A. Motivating and concerning factors were quantified out of 10 depending on perceived significance (0=insignificant; 10=very significant). A mixture of closed and Likert scaled questions were used to assess perceptions to PGT-A after treatment. Descriptive statistical analysis was undertaken using mean +/- SD.
Main results and the role of chance: 333 women were invited to participate. The response rate was 48.3% (n=161). The most common reasons to use PGT-A was advanced maternal age (n=84;35.1%) and repetitive (≥2) unsuccessful IVF cycles (n=77;32.2%). The most significant motivating factors to undergo PGT-A were to improve the probability of having a baby per embryo transfer (9.22+/-1.8) and optimise the chance of implantation (8.85+/-2.52). The most significant concerning factors identified were not having any embryos to transfer (5.76+/-3.4) and the potential for damage to be caused to embryos (5.45+/-3.1). More than three quarters (n=122;77.2%) achieved at least one live-birth using IVF with PGT-A. Two thirds (n=81;66.4%) of those who had a livebirth planned to tell their children regarding the use of PGT-A, whereas a minority (n=10;8.2%) did not intend to. The majority of participants were satisfied/very satisfied with their experience with PGT-A (n=117;73.6%), whereas 15.7% (n=25) were dissatisfied/very dissatisfied. Of those that were dissatisfied/very dissatisfied, almost two thirds (n=16;64%) did not achieve a live-birth. In those who were satisfied/very satisfied, just 8.5% (n=10) did not have a successful live-birth. More than two thirds (n=108;67.9%) would use PGT-A in future IVF cycles, and more than three quarters (n=123;77.4%) would recommend it to family and friends.

Limitations, reasons for caution: The data from this study was retrospective and from a single centre.
Wider implications of the findings: PGT-A offers an opportunity to optimise reproductive outcomes. This study highlights that, in a UK population, perceptions amongst women who undergo PGT-A as part of IVF are mostly positive. We clearly identify that women are more satisfied following treatment if a resultant livebirth is achieved.
Trial registration number: not applicable

P-455 Prospective acceptability study of a psychological online self-help intervention for individuals with an unmet parenthood goal (UPG)

B. Rowbottom[1], S. Gameiro[1]

[1]Cardiff University, School of Psychology, Cardiff, United Kingdom

Study question: How acceptable is a psychological online self-help intervention for individuals with an UPG?
Summary answer: Overall the self-help intervention was found to be acceptable and useful, providing structured support for individuals with an UPG, with minor amendments suggested.
What is known already: Individuals who are unable to achieve the family they desire, remaining childless or having fewer children than desired, have an UPG. People with an UPG can experience adjustment problems, resulting in poorer mental health and well-being. An in-depth systematic review of how people adjust to an UPG (Gameiro and Finnigan, 2017) generated the Three Task Model (3TM). This proposes three psychological mechanisms that facilitate adjustment to an UPG: acceptance, meaning making and pursuit of new life goals, leading to better mental health and well-being. To date there is no widely used evidence-based interventions for individuals facing an UPG.
Study design, size, duration: A prospective qualitative acceptability study of an online self-help intervention based on the 3TM was carried out. Twelve participants were interviewed twice: first to obtain their initial reaction to the intervention (T1); and eight weeks later to collect their perspectives of the intervention once they had engaged with it (T2). The interviews focused on assessing participant's perceptions of how acceptable (willingness to use intervention) and important/useful (perceived benefits of using intervention) the intervention is.
Participants/materials, setting, methods: Each participant was interviewed via Skype and audio recorded. At T1, participants were asked to 'think aloud' to the researcher as they engaged with the intervention for the first time. A semi-structured interview then took place, followed by a short questionnaire. The interview and questionnaire were repeated at T2 to gather participants' perspective after engaging with the intervention. Interviews were transcribed and analysed using thematic analysis.
Main results and the role of chance: The analysis resulted in 8 main themes, organized under 3 higher order themes: acceptability, importance, and other.

Themes under acceptability reflected that participants considered the intervention acceptable due to being easy to use, comprehensive and appropriate. Nearly all participants used the intervention individually, with one using it verbally with their partner. Just under half engaged with it digitally, the rest printed out the activities. Barriers to acceptability included limited digital access, poor interactivity, and unclear organisation. A minority of participants felt some sections were difficult to understand and some language was not always appropriate.

Themes under importance showed that all participants felt the intervention was useful and helpful, given that it provided a structure to organise and clarify thoughts, whilst providing guidance to move through their loss. Around two thirds of participants initially felt concerned that engaging with the intervention might be challenging. However, a majority experienced no negative effect engaging with the intervention, with three participants reporting they felt upset at times.

Themes under other highlighted that participants saw their experience of adjusting to UPG as a journey and that the intervention could facilitate this. Finally, participants thought that connecting with others is important and that the intervention can support this.
Limitations, reasons for caution: The study had a small homogeneous sample of childless married infertile women and results may not reflect the views of people who have children (but desire more), whose parenthood goals were not achieved due to other reasons (e.g., lack of partner, partner who does not want children) and of men.
Wider implications of the findings: This study shows that a psychological intervention based on the 3TM is perceived to be acceptable and useful, suggesting it successfully responds to an unmet need for support from infertile women with an UPG. Future work will focus on improving the intervention as per participants' feedback and testing its efficacy.
Trial registration number: Not applicable

P-456 Promoting fertility awareness and preconception health using a chatbot: A randomized controlled trial

E. Maeda[1], A. Miyata[2], J. Boivin[3], K. Nomura[1], Y. Kumazawa[4], H. Shirasawa[4], H. Saito[5], Y. Terada[4]

[1]Akita University Graduate School of Medicine, Environmental Health Science and Public Health, Akita, Japan ;
[2]Dokkyo Medical University, Reproduction Center, Saitama, Japan ;
[3]Cardiff University, School of Psychology, Cardiff, United Kingdom ;
[4]Akita University Graduate School of Medicine, Obstetrics and Gynecology, Akita, Japan ;
[5]Umegaoka Women's Clinic, ART Center, Tokyo, Japan

Study question: What are the effects of using a fertility education chatbot on knowledge, intentions to improve preconception behaviour, and anxiety?
Summary answer: Providing fertility education using a chatbot improved fertility knowledge and intention to optimize preconception behaviour without increasing anxiety.
What is known already: Providing fertility information through educational brochures improves fertility knowledge and changes intentions regarding childbearing, but increases anxiety. Chatbots are automatic conversation programs that are increasingly used in health education, but not yet for fertility awareness.
Study design, size, duration: A three-armed randomized controlled trial was conducted using an online social research panel in Japan in March 2019. All 927 participants were randomized and exposed to one of three materials: a chatbot (intervention group, IG), a document about fertility and preconception health (control group 1, CG1), or a document about an irrelevant topic (control group 2, CG2).
Participants/materials, setting, methods: Participants were women aged between 20 and 34 years old who hoped to have (more) children in the future. The scores for the Japanese version of the Cardiff Fertility Knowledge Scale and intention to optimize eight preconception behaviours among those who did not exhibit that behaviour were assessed immediately before and after exposure to the material. The scores for the State-Trait Anxiety Inventory and the free-text feedback provided by chatbot users were assessed after exposure.
Main results and the role of chance: Baseline characteristics were well balanced between groups. A repeated-measures analysis of variance showed significant fertility knowledge gains after the intervention in the IG (+9.1 points) and CG1 (+14.9 points), but no significant change in the CG2 group (+1.1 points). Post-test increases in the intention to take folic acid, to receive human papilloma virus vaccination, to obtain a primary obstetrics and gynaecology doctor, to take oral contraceptives, and to try to get pregnant were significantly higher in the IG than in CG2 and were similar to that in CG1. Post-test state anxiety scores (mean ± SD) were significantly lower (less anxiety) in IG (43.2 ± 9.5) than in CG1 (47.5 ± 9.5) and CG2 (46.2 ± 9.0), all $P < 0.001$. Three themes emerged from user feedback about the chatbot: technical limitations (e.g., instability of the system such as freezing, low comprehension of users' words); pros and cons of using chatbot (e.g., easy and convenient versus robotism and coolness); and experiences of learning about fertility and preconception health.
Limitations, reasons for caution: The possibility of selection bias associated with the use of a social research panel and volunteer bias toward those more interested in fertility may limit the generalizability of these findings.
Wider implications of the findings: The improvement in fertility knowledge was smaller than that of participants who read a well-written booklet but applied on a large scale may still warrant implication. Further technical development of the chatbot and exploration of personal affinity for technology is required.
Trial registration number: UMIN Clinical Trials Registry (UMIN000035736)

P-457 The age of the patient at the time of decision making is the only factor associated with the future use of conditionally blocked donors

V. Muyshond[1], A. Tolpe[1], A. De Baerdemaeker[1], K. Tilleman[1]

[1]Ghent University Hospital, Reproductive Medicine, Gent, Belgium

Study question: Which patient characteristics are predictive for further use of a sperm donor after the donor has been conditionally blocked?

Summary answer: The younger a patient is when confronted with the use of a quarantined sperm donor, the more likely she will use it for future treatments.

What is known already: Harm to a fetus via transmission of disease is considered a severe adverse reaction and needs to be reported through a biovigilance system. Karyotype screening and carrier screening for cystic fibrosis and spinal muscular atrophy are mostly performed in sperm donors and although this reduces the risk for transmission of the latter diseases, it does not diminish the risk for transmission of other conditions (Isley L. et al. 2016). When a donor child is born with a condition, sperm donors are conditionally blocked, distribution of semen restricted. Patients are notified and decisions on further use of the donor are necessary.

Study design, size, duration: A retrospective descriptive analyses was performed of sperm donors imported from EU sperm banks that were blocked in the period from 01/01/2010 to 31/12/2019. From this patient population, a subgroup was selected based on the fact that these patients had already given birth to a child of this donor or were in treatment at the moment of the blocking. This subgroup of patients had to decide to further use the donor or not.

Participants/materials, setting, methods: In total 767 patients had been treated with donors (n=196) that were subsequently blocked. Patients who had to make a decision on further use of the donor were selected. Variables like country of residence, relation type, children conceived and number of children born, age patient at time of decision and the straws left were included. Mixed model binary logistic regression and Fisher's exact test was used for statistical analysis ($p \leq 0.05$).

Main results and the role of chance: Of the total population, 292 patients were informed about the condition of the donor and a selected sample size of 184 patients had to make a decision on further use of in total 104 blocked donors. 87/184(47%) of the patients decided not to use the donor for future treatments, compared to 57/184(31%) decided to further use and 40/184(22%) have not decided yet.

At the time of decision making, patients had on average 3.2 straws left of the donor and the average age of the patient was 36.23years. Of the 292 patients, 144 made an informed decision on the further use of the donor.

More than half of the lesbian couples chose to not further use the straws (55% (42/77)), 69%(29/42) of the heterosexual couples and 64%(16/25) of the single mothers decided not to use the blocked donor in further treatments (p=NS). Binary logistic regression analysis including multiple variables showed that only the age of the patient had predictive value for deciding not to further use the straws: the average age of a patient deciding not to use the donor (av±SD 37.77±5.45) is higher compared to the average age of a patient deciding to further use the donor (av±SD 33.88±4.67) (p=0.02).

Limitations, reasons for caution: Of the selected sample size, 22% of the patients did not yet decide on the future use of the donor. There might be more intrinsic factors related to the decision making of the patients that are not included in the analysis.

Wider implications of the findings: The future use of a donor is probably related to the fact that the genetic link between future siblings is important to the parent(s), regardless of the type of relationship the patient is in. However more research into decision making of patients in these cases is very much needed.

Trial registration number: B2015/1014, amendment 2020

P-458 Discontinuation of treatment in subfertile couples with cryopreserved embryos: a multicenter study on rates and reasons of dropout.

F. Vanden Meerschaut[1], C. Blockeel[2], S. Blaiberg[3], A. Delbaere[4], A. Delvigne[5], L. Henry[6], R. Imbert[7], S. Lie Fong[8], A. Van De Vijver[9], C. Wyns[10], D. De Neubourg[11]

[1]Ghent University Hospital, Department for Reproductive Medicine, Ghent, Belgium ;
[2]Universitair Ziekenhuis Brussel, Center for Reproductive Medicine, Brussels, Belgium ;
[3]CHU St Pierre, Center for Reproductive Medicine, Brussels, Belgium ;
[4]Hôpital Erasme- Université Libre de Bruxelles, Fertility Clinic, Brussels, Belgium ;
[5]Clinique CHC Mont Légia, Centre de Procréation Médicalement Assistée, Liège, Belgium ;
[6]CHR Citadelle, Centre de Procréation Médicalement Assistée, Liège, Belgium ;
[7]Chirec-hôpital de Braine l'alleud, Centre de Procréation Médicalement Assistée, Braine l'alleud-Waterloo, Belgium ;
[8]Leuven University Hospital, Leuven university fertility center, Leuven, Belgium ;
[9]AZ Sint-Jan, Center for Reproductive Medicine, Bruges, Belgium ;
[10]Cliniques universitaires Saint-Luc, Gynécologie- andrologie et fertilisation in vitro, Brussels, Belgium ;
[11]Antwerp University Hospital, Center for Reproductive Medicine, Antwerp, Belgium

Study question: What is the dropout rate for couples with remaining cryopreserved embryos and what are the reasons for postponing or discontinuing the transfer of cryopreserved embryos?

Summary answer: The dropout rate is 9.0% in patients with remaining cryopreserved embryos. Main reasons to discontinue their treatment are psychological/physical burden and impact on work.

What is known already: In fresh ART cycles, the physical and psychological burden associated with an ART treatment is the major reason for patients to delay or even discontinue their treatment. For IVF and ICSI, rates of discontinuation vary in Europe from 17 to 70%. In addition, discontinuation of ART treatment may contribute to compromised cumulative pregnancy rates in ART. In case of the presence of cryopreserved embryos, delaying treatment is probably less frequent given the lower burden of a natural or artificially prepared frozen embryo transfer cycle. Nevertheless, no data are available regarding rates and reasons for dropout in this population.

Study design, size, duration: This multicenter study included patients from 11 Belgian fertility centers between 2012 and 2017. Patients were considered dropouts (n = 1917) in case they underwent an unsuccessful fresh ("fresh group") or cryo ("in between group") embryo transfer and did not start a subsequent cryo cycle within one year.

Participants/materials, setting, methods: An online anonymous questionnaire, aiming to explore the reasons for dropout, was developed, approved by all the ethical committees and sent to the eligible patients. An unsuccessful embryo transfer was defined by no pregnancy, a biochemical pregnancy, an ectopic pregnancy or a miscarriage <12 weeks. Exclusion criteria were cryopreservation of embryos in case of preimplantation genetic testing, fertility preservation cycles and oocyte donation cycles.

Main results and the role of chance: The overall treatment dropout rate for couples with remaining cryopreserved embryos is 9.0%. The dropout rates in the "fresh" and "in between" group are 9.7% and 8.0%, respectively. The response rate for the online questionnaire was 15.8% (n = 304/1917). The most important reasons for dropout are psychological (50%) and physical (43%) burden, the impact on work (29%), age of the woman in treatment (25%) and the impact on relationship (25%). The decision of the couple to postpone or stop treatment is influenced by external factors in only 16% of the cases. Seventy-eight percent of respondents are satisfied with the care delivered by their fertility center, and 92% would recommend the center to peers. Out of suggested improvements to the respondents, psychological support before (41%), during (51%) and after (51%) treatment, as well as lifestyle counseling (44%) and receiving digital information (43%) are most frequently selected.

Limitations, reasons for caution: As questionnaires cannot fully capture emotional responses or feelings, open answer boxes were added to the 10-point Likert scale used for some of the questions. Although the response rate is quite high compared to similar studies in the field, the results should not be extrapolated to the entire dropout population.

Wider implications of the findings: Based on our data, patients highly recommend enhanced psychological support throughout the entire ART treatment, as well as lifestyle advice and digital communication regarding information relevant for the treatment. This stresses the need to better integrate these aspects of patient centered care in our daily ART practice.

Trial registration number: not applicable

P-459 What happens in the counsellor's room? A qualitative study on the aims of psychological intervention in Italy

Z. Donarelli[1], G. Lo Coco[2], S. Gullo[2], V. Oieni[1], A. Marino[3], A. Allegra[3]

[1]Andros Day Surgery Clinic, Psychology Unit, Palermo, Italy ;
[2]University of Palermo, Department of Psychology- Educational Science and Human Movement, Palermo, Italy ;
[3]Andros Day Surgery Clinic, Reproductive Medicine Unit, Palermo, Italy

Study question: What type of intervention, methods and goals guide the Mental Health Professionals' (MHPs) work with couples in infertility counselling?
Summary answer: Although Italian MHPs' interventions follow different approaches in infertility counselling, there is a consensus on aiming to strengthen personal generativity beyond the goal of pregnancy.
What is known already: Within the context of infertility, three types of counselling are commonly adopted (implication counselling, support counselling and therapeutic counselling) and ESHRE has recognized the need for providing integrated psycho-social care by the entire staff. Although some evidence-based guidelines (which should guide psychological interventions in infertility clinics) focusing on the themes and aims of psychological counselling have been provided in recent years, there is scarce evidence on the MHP's adherence to these manuals. Moreover, there is a dearth of research on how MHPs really conduct their interventions in countries where clinics are not compelled to include MHPs in their staff.
Study design, size, duration: This was a cross-sectional qualitative study of 14 experienced MHPs who have been working in public and private infertility clinics in different regions of Italy. All professionals were individually interviewed during a five-month period from June to October 2019. Semi-structured interviews were held in Italian via Skype or phone and lasted between 60 and 80 minutes. Each interview was transcribed verbatim.
Participants/materials, setting, methods: Eighteen invitations to participate in an interview-based study were sent to MHPs and 14 agreed to participate. The interview guide was devised based on themes identified in the literature review. Data were analyzed thematically using NVivo software. A coding framework was drafted by the Principal Investigator and reviewed by the team. The entire data set was then coded by two researchers, using the coding comparison function in NVivo, reporting fair to good agreement.
Main results and the role of chance: An all-encompassing thematic description of the data set was produced. The key themes were classified under the following thematic headings: providing implication counselling to couples attending infertility treatment or facing repeated failures; the couple as the unit of counselling intervention; strengthening couples' commitment to the course of treatment.

Almost all MHPs reported facing some challenges, when working with patients who had received donated gametes, such as addressing the need to build an affective parental bond beyond genetic heritage; MHPs reported a different stance from physicians when responding to a couple's requests for advice on managing the disclosure issue, often showing a tendency to hold back their personal inclinations; moreover, most MHPs experience difficulties in responding to physicians' expectations (i.e. to obtain a quick patient response and relief from anxiety and doubts/fears, or an early evaluation of psychiatric disorders to avoid possible legal proceedings in the future).

Overall, the results suggest that MHPs would like to have greater adherence to ESHRE psycho-social routine care guidelines, with a need for further integrated care by the entire staff.

However, MHPs seem to provide counselling with common strategies, pursuing the same aims, albeit with huge differences in treatment types and settings.
Limitations, reasons for caution: The auto-selection of participants may have resulted in a sample representing those with greater clinical experience and having seen a large number of patients. Moreover, the findings may underestimate the ways in which infertility counselling is delivered outside infertility Clinics.
Wider implications of the findings: This is the first study aiming to explore the characteristics of psychological counselling from the MHPs' perspective. Although some heterogeneity across clinical approaches to patients and settings exists, MHPs do deliver counselling in general attunement with couples' needs, with efforts to break the barriers of non-integrative care.
Trial registration number: Not applicable

P-460 Reproductive choices after oocyte vitrification for age-related reasons.

J. Nekkebroeck[1], L. Leunens[1], E. Buyse[1], H. Tournaye[1], M. De Vos[1]

[1]UZ Brussel, Centrum voor Reproductieve Geneeskunde, Jette- Brussels, Belgium

Study question: What is the relational status of women who returned to the fertility clinic after planned oocyte vitrification?
Summary answer: Of women who returned to the clinic after planned oocyte cryopreservation, 48% (59/122) had found the right partner to pursue childbearing.
What is known already: The profile of women seeking planned oocyte vitrification has been quite stable during the past decade. Most women are in their mid-and late thirties, are highly educated and have a career. These women attribute their childlessness to 'not having a suitable partner' rather than to 'prioritisation of career achievements'. Hence, the main driver for planned oocyte cryopreservation is to buy more time to find a suitable partner. The return rates of these women to the fertility centre and the utilisation rate of the cryopreserved oocytes are only 7-12%. Little is known about their reproductive choices.
Study design, size, duration: In a single centre, computerized clinical data were retrieved from women who underwent planned oocyte vitrification for age related reasons between January 2009 and December 2018 (n=668) and from those who returned (n=122) to the centre to discuss their reproductive options and/or to proceed with reproductive treatment.
Participants/materials, setting, methods: We collected data from computerised clinical charts of women who applied for reproductive treatment after planned oocyte vitrification. We evaluated whether women actually started treatment, assessed their relational status (single or in a relationship) and the utilisation rate of vitrified oocytes and treatment outcome.
Main results and the role of chance: 18.2% (122/668) women returned to the fertility centre. Four were beyond the age limit of IVF in Belgium and requested transport of their oocytes to a centre abroad. For four women the cryopreservation period of 10 years had expired. Upon return to the clinic, 55 women were single and 59 had found a partner. 28.8% (17/59) of women with a partner gained information but did not proceed with fertility treatment. No less than 42% (23/55) of single women refrained from fertility treatment after counselling by a gynaecologist and psychologist. For 60.9% (14/23) of those, a genetic reference for their child was utmost important whilst only 3/23 requested an anonymous sperm donor. 60.7% (74/122) women proceeded with reproductive treatment (artificial insemination, IVF or ICSI) of which 42 had a partner and 32 were single. The utilisation rate of vitrified oocytes in women who returned was 37% (45/122). Of these 45 women who used their vitrified oocytes, 17 had an ongoing pregnancy and 4 already delivered a baby.
Limitations, reasons for caution: Patients who did not return to the centre were not included. Hence, the reproductive pathway of only a subset of women who had their oocytes cryopreserved was analysed. Follow-up of reproductive outcomes in women who did not return is required for a comprehensive appraisal of planned oocyte vitrification.
Wider implications of the findings: Almost 50% of women who returned to the fertility centre with a desire for pregnancy had found a partner. After counselling, an important subset (42%) of single women decided not to pursue single motherhood.
Trial registration number: not applicable

P-461 Impact of pre-treatment optimism on emotional health during the waiting period and after treatment results

J. Boivin[1], H. Ockhuijsen[2], R. Morey[3], A. Van den Hoogen[4], A. Domar[5], N. Macklon[6]

[1]Cardiff University, School of Psychology Cardiff Fertility Studies Research Group, Cardiff- Wales, United Kingdom ;
[2]University Medical Center Utrecht, Women & Baby Unit, Utrecht, The Netherlands ;
[3]Cardiff University, School of Psychology, Cardiff- Wales, United Kingdom ;
[4]University Medical Centre Utrecht, Birth Center Neonatology, Utrecht, The Netherlands ;
[5]Harvard Medical School, Obstetrics- Gynecology- and Reproductive Biology, Boston, U.S.A. ;
[6]London's Women's Clinic, London's Women's Clinic, London, United Kingdom

Study question: What is the association between pre-IVF success expectations and wellbeing during and after treatment?
Summary answer: Patients were more optimistic than they perceived their doctors to be but this optimism was not harmful to wellbeing during or after the pregnancy test
What is known already: Many professionals worry that excessive patient optimism at the start of IVF will negatively impact emotional reactions during treatment and after a treatment cycle. Staff report managing expectations to be a major challenge in working with patients. Patients tend to over-estimate the chance of success with treatment, even among groups with characteristics that should reduce expectations of success (e.g., older age). The effect of high optimism on wellbeing during and after treatment results is not known.
Study design, size, duration: The study comprised a secondary analysis of data from the 20-month Positive Reappraisal Coping Intervention (PRCI) randomised controlled trial (Ockhuijsen 2014). Only data from the two control groups not receiving PRCI were used in analysis. Of 1445 invited, 230 were randomised to the control groups. Participants completed questionnaires prior to treatment (Time 1, T1), 10 days after embryo transfer during the waiting period (Time 2, T2), and six weeks after embryo transfer (Time 3 T3).
Participants/materials, setting, methods: Participants were recruited from a fertility clinic if being female gender and undergoing stimulated/cryopreserved IVF/ICSI cycle. Patient optimism was measured as a difference score between their ratings (0 to 100%) on: "What do you personally think is your chance of success with your treatment cycle?" and "What do you recall your doctor telling you was the chance of success with your treatment cycle?". At T1, T2, T3 women completed the Hospital Anxiety and Depression Scale.
Main results and the role of chance: Mean personal chance of success 42.70% (SD=22.76) was significantly higher than recalled doctor chance of pregnancy 30.42% (SD=16.52) (n=157, t(156)=6.57, p < .000). The difference between self and doctor estimates decreased as doctor estimates were reported to be more optimistic (F(3, 153)=4.58, p <.004). Regression was used to examine association between patient optimism and wellbeing (anxiety, depression) during and after treatment results (controlling for pre-treatment anxiety and depression). Overall regression showed that after controlling for pre-treatment values, patient optimism was not associated with anxiety (F(1, 130)=.497, p. = 482, B=-.049 [95% CI -.041 - .020]) or depression F(1, 130)=.481, p = .489, B=-.044 [95% CI -.031 - .015]) during the waiting period. Similarly, after controlling for pre-treatment and waiting period scores, pre-treatment patient optimism was not significantly associated with post-treatment anxiety (F(1, 116)=.000, p =.993, B=-.001 [95% CI -.008 - .993]) or depression (F(1, 116)=.202, p. = .654, B=-.031 [95% CI -.020 - .032]).
Limitations, reasons for caution: Participants were in the control arms of an RCT testing a psychological intervention. Optimism was measured only in reference to what patients believed the doctor said, and not what the doctor actually said. About 36.5% (n=89) of women reported not being told a chance of pregnancy with treatment.
Wider implications of the findings: Pre-treatment optimism may be a cognitive heuristic patients use to initiate treatment that does not markedly affect wellbeing in treatment. Future research should replicate findings using doctor reports and a wider range of wellbeing and psychological measures. Fertility staff should strive for accurate expectation management but not discourage optimism.
Trial registration number: not relevant

P-462 Parent's experiences of identity-release sperm donation as adult offspring obtain donor information

A. Widbom[1], S. Isaksson[2], G. Sydsjö[3], A. Skoog-Svanberg[4], C. Lampic[1]

[1]Karolinska Institutet, Women's and Children's Health- Division of Reproductive Health, Stockholm, Sweden ;
[2]Uppsala University, Department of Public Health and Caring Sciences, Uppsala, Sweden ;
[3]Linköping University, Division of Obstetrics and Gynaecology- Department of Clinical and Experimental Medicine, Linköping, Sweden ;
[4]Uppsala University, Department of Women's and Children's Health, Uppsala, Sweden

Study question: How do parents experience and handle their adult offspring's search for identifying information about their sperm donor?
Summary answer: As parent's experiences of 'fulfilling' conditions of parenthood vary, so does their willingness to integrate the donor in the parent-offspring-donor triad.
What is known already: In Sweden, offspring conceived with donor gametes are entitled to obtain identifying information about the donor when reaching mature age. Since this law was introduced in 1985, very few of the adult offspring who are eligible to obtain donor information have exercised this right. There is limited knowledge about the experiences of parents with adult offspring following identity-release donation.
Study design, size, duration: Based on a parallel multicenter study of adult offspring who had requested and received donor information at Swedish clinics, a purposive sample of their parents were approached during 2018-2019.
Participants/materials, setting, methods: Participants were 23 parents (15 women and 8 men) with varying educational and socioeconomic background and from urban and rural areas throughout Sweden. In these families, offspring's relation to the donor ranged from no further interest, considering future contact, occasional contact and regularly meeting with the donor. Individual semi-structured interviews with parents in heterosexual couples were conducted face-to-face or via telephone, transcribed verbatim and analyzed using thematic analysis.
Main results and the role of chance: Two overarching themes were constructed as (a) Fulfilling conditions of parenthood, and (b) Parents' handling of the parent-offspring-donor triad. Conditions of parenthood, e.g. the importance of parent-offspring resemblance or importance of achieving genetic parenthood, are set by parents and society, and are to varying extents fulfilled by the parents. The extent to which parenthood is experienced as 'fulfilled' appear to further influence how the parent handles the parent-offspring-donor triad. For some, the donor is included in the family, as a new father figure, or as an extension of the family. For others, the donor is kept at a distance from the family, perceiving his intentions as non-relational, or as a threat to parenthood. I.e., as parents to varying extents are confronted with conditions of parenthood, and to varying extents 'fulfill' those conditions, they develop their lines of action to the offspring's search for identifying information about his/her sperm donor on a continuum from excluding to including the donor in the family. In cases where the donor holds more ambiguous roles, or where there is disagreement in regards to the importance of the donor between the parent and the child, friction may arise within the parent-offspring-donor triad.
Limitations, reasons for caution: Concerning transferability to other populations, it should be considered that the study was performed, and parents' experiences were given, within the context of the Swedish legislation on identity-release donation.
Wider implications of the findings: The present findings highlight the complexity of providing adult offspring access to identifying information about the donor. Parents following identity-release gamete donation may benefit from counselling and support to manage family life with varying genetic linkage within and outside the family unit.
Trial registration number: not applicable

P-463 Let's talk about it: exploring attitudes towards engagement and open discussion of (in)fertility and reproductive heath on social media

S. Tothill[1], B. Grace[1], P. Zadeh[1], D. Patel[1]

[1]University College London, Institute for Women's Health, London, United Kingdom

Study question: What is the level of engagement of users on (in)fertility and reproductive health topics two popular social media platforms?
Summary answer: Level of engagement is high, especially where celebrities are involved.
What is known already: The media remains a powerful influence on health-related behaviour, warranting further critical examination. Social media platforms like Twitter, Facebook, and Instagram have rapidly redefined the process of communication between healthcare workers, patients and public, and extremely popular way of disseminating both health information and individuals' opinions and own health experiences. Yet little is documented about social media users' levels of engagement and discussion of the topic of (in)fertility, traditionally considered a taboo subject. As of January 2020, there are 1billion Instagram users and 363million twitter users globally. This study was conducted to explore engagement on fertility topics on these platforms.

Study design, size, duration: Data from both Instagram, a photo and video-sharing social networking service and Twitter, a microblogging and social networking service were analysed, to understand the engagement of users regarding the topic of (in)fertility.
Participants/materials, setting, methods: In order to find relevant posts, Google searches were initially conducted. The most popular posts were all celebrities', which were then searched on the 2 platforms over a 2 month period, ending in January 2020 and analysed. Legitimate accounts of public figures are confirmed via 'verified' badges on Instagram and Twitter. The number of followers, likes, the topics discussed and comments were all documented, analysed and categorised into themes.
Main results and the role of chance: In the past two years, many celebrities such as Amy Schumer, Anne Hathaway, Kim Kardashian-West and Chrissy Teigen, all millennials, have openly discussed their reproductive health; specifically their fertility issues and trouble conceiving. These women have over 211 million followers on Instagram and nearly a million on Twitter, showing the incredible reach their posts have. Their latest Instagram stories and posts regarding their fertility have been liked by over 16 million people, each having thousands of comments from users showing support, advice, their own personal experiences and heartache. Topics such as IVF, Infertility, endometriosis and TTC have been hashtagged over 5 million times, with other words such as #IVFJourney, #InfertilityWarrior and #InfertilitySucks, having hundreds of thousands of posts and comments. Celebrities opening up about such intimate issues has given social media users the confidence to open up about their personal fertility struggles and realise they are not alone in their journey. Attitudes are changing across generations, Michelle Obama, only recently publicly discussed her IVF experience which happened over 2 decades ago. As Gen-Z-ers start to embark on their fertility journey, social media usage for disseminating (in)fertility information will continue rise. However, so will the risk of spreading inaccurate information.
Limitations, reasons for caution: A key methodological limitations is that findings mostly reflect views of western social media users. As use of social media continues to rise in less developed countries, further studies can provide more geographic and global representations. As not all posts include hashtags, our findings may under-represent levels of engagement.
Wider implications of the findings: As attitudes towards discussions of fertility and reproductive health continue to evolve, social media and celebrity influence will continue to be influential tools. This should be considered in the context of improving fertility awareness and effective digital health interventions based on robust scientific evidence.
Trial registration number: Not applicable

P-464 Perceived threat of infertility and women's intention to anticipate childbearing: the mediating role of personally perceived barriers and facilitators

J. Pedro[1,2], T. Brandão[3], J. Fernandes[1], A. Barros[4,5], P. Xavier[5,6], L. Schmidt[7], M.E. Costa[1,2], M.V. Martins[1,2]

[1]University of Porto, Faculty of Psychology and Educational Sciences, Porto, Portugal ;
[2]Centre for Psychology at University of Porto-, University of Porto, Porto, Portugal ;
[3]Universidade Autónoma de Lisboa Luís de Camões, CIP- Departamento de Psicologia-, Lisboa, Portugal ;
[4]University of Porto, Department of Genetics- Faculty of Medicine, Porto, Portugal ;
[5]Centre for Reproductive Genetics A. Barros, Porto, Portugal ;
[6]University of Porto, São João Hospital, Porto, Portugal ;
[7]University of Copenhagen, Department of Public Health, Copenhagen, Denmark

Study question: What are the roles of the perceived threat of infertility (severity and susceptibility), barriers and facilitators on the intention to anticipate childbearing?
Summary answer: Perceived threat of infertility affects intention to anticipate childbearing via the perception of infertility as barrier and willingness to go through fertility treatments as facilitator.
What is known already: Previous studies showed that people of reproductive age do not have adequate fertility awareness. Infertility might be partly preventable (if people try to conceive earlier in their lives) and it has been supported that the postponement of childbearing can also be partly attributable to low fertility awareness. Some intervention studies have targeted fertility awareness to promote it, but very few considered barriers, facilitators or intentions regarding individuals' reproductive plans. Studies exploring the complex relationship between the perceived threat of infertility and undertaking fertility protective behaviours are needed.
Study design, size, duration: For this cross-sectional study, childless women were invited to participate, at private gynaecology clinics and through social networks between July 2016 and February 2018. The eligibility criteria were: being involved in a romantic heterosexual relationship (≥1 year); desiring to have children in the future; being between 20-45 years of age; not having knowledge of a fertility problem, and not being actively trying to conceive for ≥12 months (or 6 months, if women aged ≥ 35).
Participants/materials, setting, methods: The sample is composed of 240 childless women committed in a heterosexual relationship who desired to have children in the future. Women completed a self-reported questionnaire including measures about their reproductive project, barriers, and facilitators to achieving their reproductive goals, and infertility' susceptibility and severity. A mediation model using PROCESS was used to test whether the relationship of the perceived threat of infertility and intention to have children earlier is mediated by barriers and facilitators.
Main results and the role of chance: Women were on average 28 years old and were committed in their relationship for an average of 6 years. Participants desired to have 2.25 children, the first one at 30.5 years old and the last at 34.5. Only 25% reported high intention to try to have children earlier than planned. Being actively trying to conceive and the number of desired children were correlated with higher intention to anticipate childbearing. For this reason, these two variables were further introduced in the model as covariates. The mediation model revealed no significant direct effect after including mediators (effect=.02; 95%CI [-.013, .044]). Two indirect effects of perceived threat of infertility on intention to have children earlier were found, through perceiving infertility as a strong barrier [estimate for indirect effect: .01 (bias-corrected (BC) 95% confidence interval (CI) = .005; .027)], and through being willing to use fertility treatment as a facilitator [estimate for indirect effect: .01 (BC 95% CI= .001; .016)]. The analysis confirmed a full mediation model and it explained 20% of the variance of intention to have children earlier.
Limitations, reasons for caution: Due to the nature of our sample and recruitment in several settings, the results need to be interpreted with caution. The cross-sectional design does not allow drawing causal directions; further longitudinal studies exploring the role of these variables are needed.
Wider implications of the findings: Perceived threat of infertility seems to be associated with the intention to anticipate childbearing. Due to lack of fertility awareness, intervention initiatives might take into account these mediators, aiming to increase the knowledge on the risk of infertility and to clarify myths that can hinder people from seeking fertility treatments.
Trial registration number: NA

P-465 Incremental validity of the Psychological Inflexibility Scale – Infertility (PIS-I)

A. Galhardo[1], M. Cunha[1], B. Monteiro[2], J. Pinto-Gouveia[3]

[1]Instituto Superior Miguel Torga- Faculty of Psychology and Educational Sciences of the University of Coimbra, Psychology- Center for Research in Neuropsychology and Cognitive and Behavioral Intervention CINEICC, Coimbra, Portugal ;
[2]Instituto Superior Miguel Torga, Psychology, Coimbra, Portugal ;
[3]Faculty of Psychology and Educational Sciences of the University of Coimbra, Center for Research in Neuropsychology and Cognitive and Behavioral Intervention CINEICC, Coimbra, Portugal

Study question: Does an infertility-related self-report measure of psychological inflexibility, the Psychological Inflexibility Scale – Infertility (PIS-I) perform better than a generic measure of psychological inflexibility?
Summary answer: The PIS-I was found to add for the prediction of infertility-related mental health outcomes as well as more general mental health outcomes.
What is known already: Psychological inflexibility (PI) has been defined as a core transdiagnostic mechanism involved in the development and maintenance of a broad range of psychological difficulties. This led to the development of several context-related measures of PI (e.g., pain, tinnitus, diabetes), besides its general measure, the Acceptance and Action Questionnaire (AAQ-II). The PIS-I was developed to assess infertility-related PI. Previous studies have suggested

that people facing infertility showed higher scores in PI,when compared to fertile couples and couples pursuing adoption. Furthermore, PI showed a mediation role in the relationship of the impact of infertility on several life areas and depressive symptoms.

Study design, size, duration: The study had a longitudinal design. Participants were recruited through the Associação Portuguesa de Fertilidade (patients' association). Inclusion criteria were age (18 years or older) and an infertility medical diagnosis. Data were collected online through self-report instruments between June and December 2018. A sub-sample of 53 participants completed the PIS-I 6 weeks after the first administration.

Participants/materials, setting, methods: A sample encompassing 313 participants (287 women and 26 men) completed online the PIS-I, the AAQ-II, the Fertility Problem Inventory (FPI), the Infertility Self-efficacy Scale (ISE) and the Depression, Anxiety and Stress Scales – 21 (DASS-21). Partial correlations, controlling for the AAQ-II, were calculated to test the PIS-I incremental validity. Moreover, hierarchical regression models were computed to address the predictive power of the PIS-I regarding infertility-related mental health outcomes and more general mental health outcomes.

Main results and the role of chance: Partial correlations results were all significant, ranging from .16 to .45, suggesting that the PIS-I accounts for important variance in several measures after accounting for the related construct of general psychological inflexibility. Concerning infertility related mental health outcomes, in the first step the AAQ-II predicted infertility-related stress (FPI) and infertility self-efficacy (ISE) with R^2values of .40 and .20, respectively. In the second step the PIS-I significantly predicted these dependent variables above and beyond the AAQ-II with increases in R^2values of .12 and .04. Regarding general mental health outcomes, in the first step the AAQ-II acted as a significant predictor for each outcome with R^2values ranging from .24 to .40. In the second step, the PIS-I also significantly predicted the depression, anxiety and stress symptoms with increases in R^2values between .01 and .05.

Limitations, reasons for caution: The online recruitment tends to recruit more educated participants, with more access to online platforms. The study was disseminated through a patients' association, limiting the inclusion of people with infertility who do not seek medical treatment. The sample included more female participants when compared to male participants.

Wider implications of the findings: Context-specific measures have proved to be valuable, allowing to capture more specified features of psychological inflexibility. The PIS-I also showed to be a useful context-specific measure of PI for people facing infertility, adding to the prediction of other mental health outcomes.

Trial registration number: N/A.

P-466 How do men want to receive information about fertility? An evaluation of a fertility campaign targeting Danish men in Copenhagen

A.S.N. Berthelsen[1], A.L.N. Gamby[1], U. Christensen[2], L. Schmidt[2]

[1]University of Copenhagen, Department of Public Health, Copenhagen, Denmark ; [2]University of Copenhagen, Department of Public Health- Section of Social Medicine, Copenhagen, Denmark

Study question: What are young men's attitudes towards the fertility campaign "How's your sperm?", and how do they want to receive fertility information in the future?

Summary answer: The young men found the campaign relevant but it had limited impact on them. They preferred fertility information through web-based venues and during school.

What is known already: It is estimated that 16-26% of the Danish population who want children, at some point in their lives, will experience infertility. In Denmark, 40% of young healthy men have decreased sperm quality, and every fifth 50-year old man is childless. The fertility awareness is limited among men. There are few fertility awareness initiatives targeting men. In October 2018, the Municipality of Copenhagen launched the campaign 'How's your sperm?' as a tool to increase fertility knowledge among men. In order to identify potential barriers for the effect of fertility campaigns targeting men, evaluation of such campaigns is needed.

Study design, size, duration: Qualitative focus group interviews with 27 men distributed in six focus groups were carried out. Furthermore, three expert interviews of the main campaign developers were conducted to identify the logic model of the intervention. Data collection took place between April and October 2019.

Participants/materials, setting, methods: The interviewed men were single or cohabiting childless men and were all residents in the Capital Region of Copenhagen. The men were between 23 to 32 years old, and almost all were university students or had a university degree. The interviews were audiotaped, anonymized and transcribed in full. Data were analyzed using qualitative content analysis following the method by Graneheim and Lundman.

Main results and the role of chance: The overall theme regarding men's attitudes towards a fertility campaign and fertility awareness in general was: 'Fertility interventions targeting men'. The subthemes were: 'Campaign communication', 'Campaign exposure and relevance for the target group', 'The sender and aim of the campaign', 'Considerations of infertility', 'Attitudes towards future parenthood', 'Fertility knowledge' and 'Future initiatives'. Overall, the campaign had limited impact on the men because they believed the campaign was not relevant to their current life situation. Furthermore, the men were confused about the aim and message of the campaign, as they thought the campaign encouraged men to have their sperm quality tested. The men also criticized the campaign for making a link between sperm quality and masculinity. They recognized the importance of knowledge about reproductive health but they wanted more accurate information about fertility and risk factors for infertility. According to the men, future initiatives should prioritize accurate dissemination of fertility information in web-based venues. In addition, the men suggested that fertility information should be a mandatory part of the (sexual) education in upper-secondary schools and/or university level.

Limitations, reasons for caution: Most of the interviewed men had a university degree and wanted more facts instead of humor in the campaign, hence our results may not be directly transferred to a similar age group in the general population.

Wider implications of the findings: Our study contributes to the understanding of how to communicate future fertility awareness campaigns and initiatives. This may be useful in the process of increasing the fertility awareness in the population.

Trial registration number: N/A

P-467 Taking fertility for granted – A qualitative exploration of fertility awareness among young, childless men in Denmark and Sweden

G.M. Malling[1], L. Schmidt[1], T. Pitsillos[2], K. Hammarberg[3], T. Tydén[2], B. Friberg[4], I. Toftelund Jensen[1], S. Ziebe[5]

[1]University of Copenhagen, Department of Public Health, Copenhagen K, Denmark ; [2]University Hospital Uppsala, Department of Women's and Children's Health, Uppsala, Sweden ; [3]Monash University- Melbourne, Jean Hailes Research Unit- School of Public Health and Preventive Medicine, Melbourne, Australia ; [4]Lund University, Molecular Reproductive Medicine- Department of Translational Medicine, Malmö, Sweden ; [5]Copenhagen University Hospital- Rigshospitalet, The Fertility Clinic, Copenhagen, Denmark

Study question: How do young childless men in Denmark and Sweden reflect on their fertility, view fertility treatment, and how aware are they about infertility risk factors?

Summary answer: Most men had limited knowledge about factors that can impair fertility, presumed they were fertile, and were positive towards fertility treatment if they needed it.

What is known already: Almost all men in the Nordic countries want to become fathers and want at least two children. Previous qualitative interview studies with childless men aged 21-46 have shown that most men expect to become fathers when they want to. Further, recent reviews have concluded that men around the world have limited knowledge about fertility and factors that can diminish it. Most previous studies about fertility knowledge and attitudes among men have been based on quantitative methods using questionnaires with fixed-choice response options.

Study design, size, duration: This qualitative study assessed knowledge about fertility and attitudes towards fertility treatment among childless men in Denmark and Sweden in their last year of university education or vocational

training. In total, 17 Danish and 12 Swedish male students were interviewed. The interviews were conducted between February and September 2017 and ranged between 30 and 90 minutes, with an average length of one hour.

Participants/materials, setting, methods: Participants were recruited face-to-face, through postings on their educational institutions, and by snowball methods. Inclusion criteria were: being male, childless, aged 20-30 years and in the last year of education. The interview guide included questions probing participants' perceptions about their own fertility, fertility treatment, and fertility risk factors. The interviews were recorded and transcribed and the content analysed thematically.

Main results and the role of chance: Study participants were on average 25 years old (range 20-30). The analysis revealed the following themes: 1) Hope and Fear; 2) Parenthood and Involuntary Childlessness; 3) Fertility Treatment – A great Alternative; 4) Factors Meant to Influence Fertility; and 5) Uncertainty. Few participants had considered their own fertility but most were optimistic about their ability to become a parent in the future. Among those who that had thought about their fertility, hope and fear were the most prominent emotions. Most men were positive towards and would consider having fertility treatment if they experienced fertility problems. The young men had limited knowledge about factors that can potentially impair male and female fertility. On average each participant mentioned three different factors they believed influence male and female fertility either positively or negatively. However, their responses often entailed uncertainty, illustrated through words like 'uh', 'I think', 'maybe', and 'could be'. None of the men mentioned sexual transmitted infections (STIs) as a risk factor for infertility but most appeared aware of the effect of increasing age on fertility.

Limitations, reasons for caution: Participants' responses to the question about factors that affect fertility may not reflect all aspects of their knowledge on the topic. Further, men who do not want children may have been less likely to participate than men who want children.

Wider implications of the findings: This study highlights the need for educational strategies to improve young men's knowledge about fertility and the factors that influence it, particularly about the potential adverse effect of STIs on fertility. Men also need to be aware that there is no guarantee of success with fertility treatment.

Trial registration number: N/A

P-468 The infertility and fertility treatment experiences of Orthodox Jewish women in London

A. Vaz Mouyal[1], Z. Gurtin[1], J. Leach Scully[2], J. Harper[1]

[1]University College London, Institute For Women's Health, London, United Kingdom ;

[2]Innovation Institute UNSW, John Goodsell Building, Sidney, Australia

Study question: What are the infertility experiences of 26 British Orthodox Jewish women?

Summary answer: Infertility challenges the way Orthodox Jewish women understand their lives and identities. It further impacts their relationships with their Rabbis, partners, families, friends and communities.

What is known already: Procreation has a very important place within Orthodox Jewish communities, therefore infertility presents a particularly severe difficulty to Orthodox Jewish women. Jewish law tends to permit the use of assisted reproductive technologies (ART). Several studies on the use of ART in Israel have been carried out. Kahn's work shows the difficulties single Jewish women face when seeking solo motherhood in a society that honours motherhood and family life (Kahn, 2000), while Ivry (2010) describes the koshering of medical care in rabbinically mediated fertility treatment. The research presented here is the first in the field carried out in the UK.

Study design, size, duration: A total of 26 Orthodox Jewish women who had experienced infertility were interviewed. All interviews were conducted between 2017 and 2018. Women were recruited via adverts in local Jewish magazines, Facebook groups and via snowballing. The study design resulted from previous research carried out in Israel and the UK as part of PhD research.

Participants/materials, setting, methods: Semi-structured qualitative interviews were undertaken. The women were mostly interviewed in their houses. Interviews were conducted in English and lasted between an hour and an hour and half. All were transcribed verbatim and any identifiable information was changed to protect the women's identity. Qualitative descriptive methods alongside thematic analysis were used to express and discuss the data collected from the interviews.

Main results and the role of chance: Four main themes were identified: my destiny, my Rabbi, my relationships and my identity. ***My destiny*** focused on the view women had of God as "controller" and "giver" of their infertility. ***My Rabbi*** introduced the deep relationship that exists between some Orthodox Jewish women and their Rabbis, highlighting the way Rabbis are viewed as representatives and messengers of God. ***My relationships*** explored the effect of infertility on the women's relationships with their partners, families, friends and communities. ***My identity*** indicated the way women internalize their infertility due to their feelings of isolation, loneliness and estrangement from their familiar culture, community and beliefs. It became apparent that for some women, who did not feel fulfilled as women unless they were able to be a mother, all sense of self and identity slowly breaks down.

Limitations, reasons for caution: Due to the small sample size and limited geographical spread, the findings cannot claim to be representative of the Orthodox Jewish community of the UK. The lead author's own Jewish status could have influenced the participants' responses and/or the analysis, although involvement of other authors mitigated this.

Wider implications of the findings: This research gives useful insight into an underresearched population. Its findings offer guidance to medical professionals, counsellors, policy makers, and religious leaders. Other Orthodox Jewish women may be helped by knowing they are not alone. The findings suggest that other underresearched populations could experience distinctive difficulties with infertility and ART.

Trial registration number: n/a

P-469 Posttraumatic growth (PTG) in women with a long-standing experience of involuntary childlessness

G. Duraskova[1], B. Peterson[2]

[1]psychologist, Motol University Hospital, Prague, Czech Republic ;

[2]professor Crean College of Health and Behavioral Sciences, Chapman University, California, U.S.A.

Study question: What aspects of posttraumatic growth (PTG) are found in women with a long-standing experience of involuntary childlessness?

Summary answer: Women developed deeper self-recognition and greater humility, they reassessed their existing relationships, strengthened their partnerships, and experienced changes in their life philosophy.

What is known already: "The term posttraumatic growth refers to positive psychological change experienced as a result of the struggle with highly challenging life circumstances" (Calhoun & Tedeschi, 2004). For women experiencing infertility, research mainly focuses on the description of negative psychological factors with only few published reports mentioning positive personality development in conjunction with the infertility experience (Lee at al., 2009; Daniluk, 2001). The lack of studies on PTG in women diagnosed with infertility represents a gap in the literature which limits our knowledge about the potential positive implications that may result from efforts to cope with this stressful life challenge.

Study design, size, duration: The study used a qualitative design interviewing 24 women with a long-standing experience of primary infertility. In-depth semi-structured interviews were conducted in the Czech Republic during 2016 and lasted an average of 53 minutes. Interviews aimed to further understand the possible experiences of PTG in study participants. Participants shared both positive and negative aspects of the infertility experience. Data were analyzed using thematic analysis.

Participants/materials, setting, methods: Invitations to participate in the study were sent to 28 women who experienced involuntary childlessness (purposive selection). The final sample consisted of 24 women (4 declined to participate). The average age of participants was 37,3 years, and their average length of experience with infertility was 6,1 years. Women were asked how infertility affected/changed their partner relationships, sexual life, job, future plans, attitude to children/values/faith, and leisure time.

Main results and the role of chance: Five main themes of posttraumatic growth were identified: greater humility, deeper self-recognition, overall reassessment of relationships, changes in philosophy of life, and strengthening partnership. The experience of infertility taught these women to have more respect to life, reassess and find their personal value, recognize their inner strength, be more emphatic, develop their spiritual lives, and be responsible for their own

health. Women connected these changes with their experience of infertility. Without a control group of participants, we cannot exclude the possibility that women may have experienced these changes without experiencing infertility.
Limitations, reasons for caution: The study findings are limited by the qualitative study design, sampling procedure, and number of participants. Because of this, we cannot generalize our results for all women with long-term infertility experience. In addition, PTG is a subjective phenomenon and is limited by retrospective self-report and reflection.
Wider implications of the findings: The findings of our qualitative study show what aspects of PTG are experienced by women experiencing infertility. Medical and mental health providers can use our findings to facilitate the PTG process in patients. Because the study only sampled women, it is unknown if/how PTG is experienced by men.
Trial registration number: not applicable

P-470 The importance of the "family clock": Women's experience of fertility decision-making 6 years after attending the Fertility Assessment and Counselling clinic

E. Koert[1], R. Sylvest[1], I. Vittrup[2], H.W. Hvidman[2], K. Birch Petersen[3], J. Boivin[4], A. Nyboe Andersen[2], L. Schmidt[1]

[1]University of Copenhagen, Section of Social Medicine- Department of Public Health, Copenhagen, Denmark ;
[2]Copenhagen University Hospital - Rigshospitalet, Fertility Clinic, Copenhagen, Denmark ;
[3]The Fertility Partnership, Fertility Clinic, Copenhagen, Denmark ;
[4]University of Cardiff, School of Psychology, Cardiff, United Kingdom

Study question: What is women's lived experience of making fertility decisions in the six years after receiving personalized fertility education, assessment and counselling?
Summary answer: Women's "family clock" (preferred start time, number and spacing of children) was an important aspect of their lived experience of fertility decision-making.
What is known already: Research demonstrates that fertility education is needed to support informed and satisfying fertility decision-making. The Fertility Assessment and Counselling (FAC) clinic in Copenhagen, Denmark (opened in 2011) is a personalized fertility education, assessment and counselling intervention aimed at increasing knowledge through provision of general fertility information and a clinical examination and evaluation of personal risk factors (Hvidman et al., 2015). A one-year follow up study of women attending the FAC clinic found that the intervention impacted women's fertility decisions and was a catalyst for change in their lives. Additional long-term follow-up is needed to explore women's experiences in detail.
Study design, size, duration: A qualitative 6-year follow-up study with 24 women who attended the FAC clinic between January and June 2012. Women were interviewed in person between February and March 2018 at Rigshospitalet, Copenhagen, Denmark. Interviews ranged between 60 to 94 minutes (average 73 minutes) and were conducted in English.
Participants/materials, setting, methods: Invitations were sent to 141 women who attended the FAC clinic in 2012. Ninety-five women opened the invitation, 35 were interested in participating, 25 interviews were booked and 24 interviews held. Interviews were semi-structured and examined the women's perceptions and experience of the intervention six years after attending. This abstract focuses on data specific to the women's lived experience of making fertility decisions after attending the FAC clinic. Data was analyzed using thematic analysis.
Main results and the role of chance: At the time of the follow-up interview, women were on average 39.5 years old (range 31-45). Ten were single and 14 were partnered. The majority (21 women, 88%) became parents in the six years after attending the FAC clinic, with almost half (9 women) starting to try to become pregnant in the first year.

The women's "family clock" played an important role in their fertility decisions – beliefs regarding when to start trying to become pregnant, their preferred number of children and spacing between them. All who tried to become pregnant had given birth to at least one child. However, all of the women with one child regretted that they had "run out of time" and not achieved their desired family size (two or three children), commonly attributed to their age, age-related fertility decline, and/or single parenthood.

Women described feeling empowered to make informed decisions about their fertility after attending the FAC clinic. However, when considering a second pregnancy several years later, women experienced the pressure of time and their age and uncertainty about their fertility. They wished for repeated counselling and advice to aid decision-making after a first pregnancy, or if several years had passed since attending the FAC clinic.
Limitations, reasons for caution: There may be a selection bias given that participants were self-selected from women who attended the FAC clinic with 25% expressing an interest in the study after receiving an invitation. That said, the data from 24 women presented a broad spectrum of fertility decision-making experiences and trajectories.
Wider implications of the findings: When providing education and guidance to women regarding future fertility plans, consideration of their "family clock" should be included so women can make informed fertility decisions about timing of pregnancy and achieve all their family goals. Additional counselling and advice after pregnancy or several years have passed may be warranted.
Trial registration number: Not applicable

P-471 What guidelines should be followed for subfertile patients to achieve spontaneuos pregnanycy? A randomized controlled trial of couples trying to conceive

M. Martins, M.Sc.- Ph.D.[1,2], J. Fernandes[2], J. Pedro[1,2], P. Xavier[3,4], A. Barros[4,5], E. Costa[1,2], L. Schmidt[6]

[1]University of Porto, Faculty of Psychology, Porto, Portugal ;
[2]University of Porto, Centre for Psychology at University of Porto, Porto, Portugal ;
[3]Porto Clínica, Reproductive Endocrinology, Porto, Portugal ;
[4]University of Porto, Faculty of Medicine, Porto, Portugal ;
[5]Centre for Reproductive Genetics A. Barros, Reproductive Endocrinology, Porto, Portugal ;
[6]University of Copenhagen, Public Health, Copenhagen, Denmark

Study question: Do educational interventions on timing intercourse improve psychosocial adjustment and pregnancy rates in unselected subfertile individuals over the course of 12 months?
Summary answer: No significant psychosocial adjustment differences were found between groups over one year. However, the 'fertile window' monitoring group achieved a higher pregnancy rate than CG.
What is known already: It is well-know that general knowledge on fertility and fecundity is low. Recent evidence also suggests that both patients and professionals lack confidence in natural conception or expectant management and that education is needed. There is solid evidence that that time to pregnancy can be significantly shortened by targeting the fertile period with cervical mucus monitoring or the use of ovulation predictor kits. However, reproductive care guidelines recommend instead the practice of intercourse every other day for being less stressful to the couple. At present, there are no studies comparing the recommendation of these two strategies against a control group.
Study design, size, duration: We conducted a prospective, double-blind, three-arm parallel RCT, recruiting between July 2016 and June 2018. Subjects (n=414, 263 women) were randomly allocated into 'fertile window monitoring' (FWM, n = 137), 'every other day' (EOD, n = 124), and control group (CG, n = 153), and were assessed before (T0), 4-weeks (T1), 6-months (T2), and 12-months (T3) after the intervention. Interventions consisted of short animated videos addressing fecundity knowledge and the correspondent trying-to-conceive (TTC) strategy.
Participants/materials, setting, methods: Subjects were recruited via social media advertising and brochures with an accompanying link to the survey, or via gynecology and fertility clinics, where patients received tablet PCs with headsets. Individuals trying to conceive and in a marital relationship for > 1 year were enrolled in the RCT, and completed self-report questionnaires measuring psychosocial adjustment besides a specific questionnaire. Mixed models ANOVA tested effects on psychosocial variables, and OR and RR were estimated for pregnancy rates.
Main results and the role of chance: Participants were in their thirties (33.12±4.46), in a relationship for around eight years (8.81±5.19) and were TTC for around two years (2.23±2.53). One participant gave-up TTC at T1, and 331 were lost to follow-up. Eighty-three subjects completed all four moments and were included in the longitudinal analysis having psychosocial adjustment as DVs. Baseline characteristics were well balanced between groups. No significant

interaction or main effects of the interventions were found for stress (PSS), depression, anxiety (HADS), and sexual functioning (FSFI/IIEF). Results remained non-significant after controlling for pregnancy (P > .05). Amongst the 263 women, 55 reported not being pregnant at follow-ups and 46 reported a pregnancy. Of these, fourteen had a positive pregnancy test after IVF/ICSI and were excluded from analysis. The final sample for pregnancy rate analysis included 101 women. Individuals in the FWM group had significantly higher pregnancy rates than those in the CG (60.7 vs 35.9%, P = .038); OR 2.8 (95% CI 1.058-7.415). There were no differences in pregnancy rates between the EOD and CG subjects, nor between EOD and FWM.

Limitations, reasons for caution: This study is prone to bias as both undiagnosed and individuals in fertility treatment were included. The analyses did not include the monitoring of the use of the TTC strategy and did not use clinical pregnancy as an outcome.

Wider implications of the findings: Results show that the EOD strategy does not lead to better adjustment nor less stress, and hence guidelines need to be reviewed. Further studies should use large samples of untreated couples TTC<12 months to confirm the potential of FWM education as a tool to empower the self-management of reproductive health.

Trial registration number: Study partially funded by the Portuguese Foundation for Science and Technology [FCT PhD grant SFRH/BD/103234/2014]

P-472 Sleep - an underrated fertility booster? A questionnaire survey on the pattern of sleep among IVF patients and their reproductive outcome

R. CHELLADURAI[1], G. Pm[1], G.D. Ss[1], H. Vaidyanathan[1]

[1]SIMS Hospital, OBG and IVF, Chennai, India

Study question: Evaluating the impact of quality and quantity of sleep on fertilisation rate and clinical pregnancy rate in patients undergoing IVF in SIMS hospital,Chennai,India

Summary answer: Fertilization rates and clinical pregnancy rates were significantly higher among patients with PQLI (Pittsburg sleep quality index) score of less than 9 (no/mild sleep difficulties)

What is known already: High levels of psychological stress, anxiety, insomnia and depression have been reported in IVF patients.Sleep disturbance is a frequent but underrated symptom in IVF patients and aggravate the existing psychological problems .Secretion of Melatonin ,the hormone responsible for improving quality of oocytes by acting as an antioxidant, is increased during sleep. Also,sleep promotes pulsatility of gonadotrophins and the sleeP-wake cycle maintains the normal hormonal balance. However in most places, sleep disturbance is disregarded and ignored even during routine psychological counselling sessions. It remains an under-investigated modifiable target that may provide a non-pharmacological ,cost-effective method to improve IVF outcome.

Study design, size, duration: A questionnaire survey conducted at SIMS hospital Chennai between Jan 2017-Dec 2019. A total of 196 women undergoing IVF were given the Pittsburg sleep quality index questionnaire on the day of stimulation to evaluate their sleeping patterns in the previous month by measuring hours of sleep , latency, having bad dreams disturbing sleep, snoring, sleep disturbances like somnambulism ,whether requiring medications to induce sleep and job related sleep disturbances like shift-work or part-time.

Participants/materials, setting, methods: Patients were divided into three categories on the basis of PQLI: (A)a total score of "5" or less indicated no sleep difficulties; '(B)'6-8" indicated mild difficulties; '(C) '9" or more indicated severe difficulties. Fertilisation rates and clinical pregnancy rates were compared between the 3 groups.

Normal responders undergoing stimulation using Antagonist protocol were included.

Exclusion criteria included poor and hyper responders, male factor, female partner age >35 years, endometriosis, PID, using Donor gametes.

Main results and the role of chance: Based on the collected responses to the above questions, a statistical comparison of ART outcomes, in three PSQI categories, was performed by Mann-Whitney U test and chi-square test. Logistic regression analysis was subsequently conducted to assess the association of the conditions in the questions with ART outcomes, as represented by fertilization rates and clinical pregnancy rates.

Clinical pregnancy was determined by observation of a gestational sac with fetal heart beat by transvaginal ultrasound at 6 weeks of pregnancy. Fertilization rate (FR) was percentage of transformation of micro injected oocytes into two pronuclei.

The number of patients in each group were A(127/196) B(39/196) and C(30/196)

The average hours of sleep in the 3 groups were 7.9 hours,7.3 hours and 6.3 hours

Fertilization rates in the Groups A,B and C were 68%,62% and 46% , and clinical pregnancy rates 44%,39% and 23% respectively with a significant p value of <0.001.

Also it was found that the prevalence of co-existent psychological issues like anxiety, depression and OCD were higher among the C group with severe sleep difficulties. 6 of them required psychotherapy . All members in group C were advised to attend our classes on YOGA and meditation.

Limitations, reasons for caution: Though the prevalence of Insomnia and sleep disturbances are common, no major studies have been done on their impact on reproductive outcome in ART. Studies using larger population and multicentric trials is necessary .

Although the outcomes were adjusted for confounding factors, some unknown confounders may affect the outcomes.

Wider implications of the findings: The importance of sleep on reproductive outcome must be reiterated and insomnia ,if detected must be treated aggressively. Simple methods such as yoga,meditation and psychological support from family and peers would go a long way in helping these women cope with the stress of anxiety and insomnia during IVF treatment.

Trial registration number: not applicable

P-473 Responses to and communication about infertility among couples treated at fertility clinic: Implications for depressive symptoms

T. Matitashvili[1], K. Slauson-Blevins[2], A. Greil[3], S. Bocca[1]

[1]Jones Institute for Reproductive Medicine- Eastern Virginia Medical School, Reproductive Endocrinology and Infertility, Norfolk, U.S.A. ;
[2]Old Dominion University, Department of Sociology and Criminal Justice, Norfolk, U.S.A. ;
[3]Alfred University, Division of Social Sciences, Norfolk, U.S.A.

Study question: Do couple's responses to infertility depend on the source of the infertility and how do responses to infertility affect one's own and partner's depressive symptoms?

Summary answer: Generally couples responses to infertility are not influenced by the source of the problem. One's own responses are typically associated with one's own depressive symptoms.

What is known already: Most studies about psychological responses to infertility have used the individual rather than the couple as the unit of analysis. Several studies have reported on the coping strategies employed by each partner in an infertile couple. Less, however, is known about the associations between difference in perceptions of the experience of infertility and communication and each partner's well-being. In addition, a number of studies have examined whether there is difference in the experience of infertility depending on which partner is being diagnosed with infertility, but, so far, no clear conclusion can be drawn.

Study design, size, duration: This was a cross-sectional study administered by paper questionnaire. Both partners of infertile couples attending a fertility clinic filled out identical questionnaires. The questionnaires included background information, reproductive history, measures of life satisfaction and depressive symptoms, and scales measuring perceptions of various aspects of the infertility experience. Information about medical diagnosis was obtained from medical records. The study involved 156 men and women, including 58 couples, who are the main focus of this paper.

Participants/materials, setting, methods: Study received IRB approval from Eastern Virginia Medical School and Old Dominion University. Patients who attended the fertility clinic from December 2013 to April 2015 were asked to fill out a questionnaire containing 161 questions. Validated scales were constructed measuring importance of children, communication about fertility, perceived fertility-specific relationship power and fertility-specific sexual satisfaction. Results were analyzed via paired-samples t-tests, one-way ANOVAs, mixed ANOVAs, and OLS regression. $P < 0.05$ was regarded as statistically significant.

Main results and the role of chance: Women reported higher levels of importance of children and fertility-related sexual problems than their partners. Men reported higher general relationship satisfaction, fertility-specific relationship satisfaction, and perceived fertility-specific relationship power than their partners. With one exception, scale scores were not associated with which partner had a fertility problem. Women scored higher than their partners on the importance of children scale except among couples with infertility of unknown cause. For women, higher scores on importance of children, satisfaction with fertility–related communication and fertility-related sexual problems were associated with higher levels of one's own depressive symptoms. For men, higher scores on satisfaction with fertility–related communication, perceived fertility-related power, fertility-related relationship satisfaction, general relationship satisfaction and fertility-related sexual problems were associated with higher levels of one's own depressive symptoms. For women, fertility-related sexual problems was associated with higher levels of partner's depressive symptoms. For men, higher scores on importance of children was associated with higher levels of partner's depressive symptoms. The higher women score on the importance of children compared to their partners, the higher their level of depressive symptoms. The higher men score on fertility-related sexual problems and satisfaction with fertility-related communication, the higher their level of depressive symptoms (all $P < 0.05$).

Limitations, reasons for caution: Because study was cross-sectional, it is impossible to make causal inferences. The findings of this study are limited in generalizability as all patients were recruited from a single site and small sample size prevented us from including more variables in each analysis.

Wider implications of the findings: These findings have important implications for counseling infertile couples. It is especially important to note that relationship satisfaction and satisfaction with communication does not always have a positive effect on well-being. Fertility-related sexual problems appear to have implications for well-being among both men and women.

Trial registration number: n/a

P-474 Luteal granulosa cells from women undergoing a stress management program during ICSI cycles exhibit lower mitochondrial DNA levels: a randomized clinical trial

G. Raad[1], J. Tanios[2], N. El Salibi[3], S. Kerbaj[4], F. Shamas[5], J. Azouri[6], Y. Mourad[7], C. Fakih[8]

[1]Al Hadi Laboratory and Medical Center, ivf, Beirut, Lebanon ;
[2]Lebanese University, Master In Cognition- Behavior- and neuroscience, Fanar, Lebanon ;
[3]King Abdullah University of Science and Technology KAUST, Counseling Center, Saudi Arabia, Saudi Arabia ;
[4]Lebanese University, Master in psychosomatic therapy, Fanar, Lebanon ;
[5]Al Hadi Laboratory and Medical Center, Molecular Biology, Beirut, Lebanon ;
[6]Mount-Lebanon Hospital Lebanon, Azoury IVF clinic, Hazmieh, Lebanon ;
[7]Al Hadi Laboratory and Medical Center, Medical Laboratory, Beirut, Lebanon ;
[8]Al Hadi Labratory and Medical Center, Ivf, Beirut, Lebanon

Study question: What impact does stress management have during intracytoplasmic sperm injection (ICSI) cycles on mitochondrial DNA (mtDNA) levels in luteal granulosa cells?

Summary answer: Women undergoing stress management during ICSI cycles had lower mitochondrial DNA levels in their luteal granulosa cells compared to the control group.

What is known already: ICSI cycles were demonstrated to be psychologically stressful for most couples. In parallel, growing evidence supports the implication of mitochondria in the stress response. Furthermore, mitochondria are considered as potential regulators of oocyte- granulosa cells cross-talk. However, the effect of psychological stress during ICSI cycles on mtDNA levels in luteal granulosa cells and subsequent oocyte competence was never fully investigated nor well understood.

Study design, size, duration: A randomized controlled trial, with pre-test-posttest experimental design was conducted on 60 couples undergoing ICSI cycles, between May 2018 and May 2019, at Mount-Lebanon hospital. On the first day of ovarian stimulation, the couples were randomized using sealed opaque envelopes into control group (n=30) and stress management group (n=30). The physicians and embryologists were blinded to the allocation. The researchers provided stress management consisting of educational sessions about the treatment and relaxation techniques.

Participants/materials, setting, methods: Literate couples with a female partner < 38 years old, using their fresh gametes, and without psychiatric disorders were included. Stress levels were assessed on the first day of ovarian stimulation and on the day of embryo transfer using the Perceived Stress Scale (PSS 10). Luteal granulosa cells were collected on the day of oocytes pick-up. Granulosa vitality and morphology were evaluated and mtDNA levels were quantified using Ion Torrent next generation sequencing technology.

Main results and the role of chance: During the first day of ovarian stimulation, statistically significant higher scores of PSS (PSS initial) were detected in women of the study group (23.1 ± 5.1) compared to the control group (19.3 ± 7.01) ($p < 0.05$). However, ΔPSS (PSS initial – PSS on the day of transfer) showed significant reduction in perceived stress in women of the study group compared to the control group ($p < 0.01$). At the ovarian level, trypan blue staining indicated that the percentage of viable granulosa was significantly higher in the study group compared to the control group ($p < 0.05$). In addition, a statistically significant higher percentage of granulosa cells with normal morphology were detected in women of the stress management group compared to the control group ($p < 0.05$). Furthermore, mtDNA levels were significantly higher in luteal granulosa cells of control group compared to the study group ($p < 0.05$).

Limitations, reasons for caution: The study excluded subjects with severe infertility factors. Consequently, the efficiency of this stress management program should be also tested in these subjects before automatically generalizing it to all infertility cases.

Wider implications of the findings: This stress management program seems to be able to reduce psychological stress among IVF patients thus improving their mental health. It also influences the ovarian physiology through the granulosa cells. Hence, further investigations are needed to evaluate whether applying this program in clinical settings could affect other ICSI related outcomes.

Trial registration number: LBCTR2019101289

P-475 Seeking pregnancy through assisted reproduction technology treatments (ART): A comparison between couples receiving donated gametes and couples using their own gametes

F. Andrei[1], P. Salvatori[1], L. Cipriani[2], G. Damiano[2], N.C. Rossi[1], E. Porcu[2]

[1]University of Bologna, Department of Psychology, Bologna, Italy ;
[2]Infertility and IVF Unit, University of Bologna-Sant'Orsola Hospital, Bologna, Italy

Study question: What are the psychological differences between couples waiting for gametes donation and those using their own gametes, considering previous ART attempts and cause of infertility?

Summary answer: Differences in infertility self-efficacy were found between the two groups, while no significant differences with regards to psychological symptoms and quality of life emerged.

What is known already: Involuntary childlessness often produces overwhelming feelings of anger, shame, grief, and guilt. For those couples who decide to undergo ART, such emotional reactions may persist, if not worsen, throughout the different phases of the treatment. Higher levels of psychological distress and lower levels of quality of life seem to affect especially couples who experienced repeated failures. Additionally, the only published study comparing couples by the severity of infertility shows that couples who use their own gametes seem to have higher depression, anxiety, infertility stress, and infertility-related sexual concerns than couples awaiting for gametes donation.

Study design, size, duration: Data were collected cross-sectionally from October 2018 to October 2019. One hundred seven couples seeking treatments to conceive took part to the study.

Participants/materials, setting, methods: Data were collected from 107 couples at their first access to the Infertility and IVF Unit of the Sant'Orsola Hospital, University of Bologna (Italy). During a first consult, clinical information were recorded and the Infertility Self-Efficacy Scale, Fertility Quality of Life, and Symptom Check-List were given to participants. During a second consult, couples were placed on a waiting list for either gametes donation (n=42) or IVF with their own gametes (n=65), and questionnaires were returned.

Main results and the role of chance: Univariate ANOVAs showed a significant interaction between previous ART attempts (yes/no) and awaited treatment (IVF with gametes donation/IVF with the couple's own gametes) with regards to infertility self-efficacy. Couples who used their own gametes showed higher levels of self-efficacy if they were at their first ART attempt compared to those who already tried to conceive through ART without succeeding. Gametes receiving couples showed an opposite pattern ($F = 6.54$, $P < .01$). With regards to quality of life, we found a main effect of the variable previous ART attempts, showing that couples who already underwent ART treatments had overall lower levels of quality of life ($F = 2.68$, $P < .01$). No differences were found with regards to levels of anxiety, depression and anger/hostility (all $Ps > .05$).

Limitations, reasons for caution: The sample size and the cross-sectional design allowed us to draw conclusions only about the associations between study variables. We could not include additional relevant variables, such as time from infertility diagnosis and parity, which may have affected our results.

Wider implications of the findings: The differences between gametes receiving couples and couples who used their own gametes in self-efficacy may be determined by both the difficulties each couple face during ART treatment and the infertility history. Shifting to gametes donation may give new hope to those couples who experienced repeated failures with ART.

Trial registration number: CE: 273/2018/Sper/AOUBO

P-476 Factors associated with decision regret following oocyte cryopreservation for diminished ovarian reserve and/or age-related fertility decline

T.A. Boza[1], A.P. Çil[1], L.S. Karakis[2], R. Abalı[2], M. Ceyhan[1], E.G. Aksakal[1], M. Bahçeci[2], B. Urman[1]

[1]American Hospital, Womens' Health Unit, Istanbul, Turkey ; [2]Bahceci Health Group, IVF Centre, Istanbul, Turkey

Study question: What are the factors associated with decision regret following oocyte cryopreservation (OC) in women with diminished ovarian reserve (DOR) and/or age-related fertility decline?

Summary answer: The perception of inadequate emotional support during the procedure and low expectations regarding its efficacy were associated with increased decision regret.

What is known already: Recently we published our data on cycle characteristics and reproductive outcomes of women with DOR undergoing OC. Since cycle cancellation rates are high and reproductive outcomes are poor in such patients, we aimed to counsel these patients about their expectations of OC and determine factors which may be associated with future decision regret. Decision regret is considered as an overall indicator of quality of health decisions. Although there are a few studies on decision regret following elective OC in women with normal ovarian reserve, no studies to date have addressed factors associated with decision regret in women with DOR.

Study design, size, duration: A cross sectional survey study was conducted involving 552 women with DOR and/or age-related fertility decline who underwent OC in two private ART centers (American Hospital and Bahceci IVF Centre) from 2015 to 2019. Following IRB approval, women were contacted by phone and asked whether they would be willing to participate in a standardized online questionnaire to evaluate decision regret, their current relationship status, satisfaction level with the procedure, and their attitude towards future childbearing.

Participants/materials, setting, methods: Of the 552 women who were contacted, 468 accepted the invitation, and 162 (34.6%) responded to the survey. The primary outcome of the study was decision regret measured using the validated Decision Regret Scale (DRS). The associations between decision regret and level of patient satisfaction, reproductive expectations and desire for parenthood were assessed. DRS was interpreted as follows: 0: no regret; 1-25: mild regret, and 25-100: moderate to severe regret.

Main results and the role of chance: Mean age at the time of OC and survey submission was 37.8±4.6 and 40 ±1.0, respectively. The total number of oocytes cryopreserved was <10 in 70% of women. The average follow-up time interval from OC to survey submission was 2.5 years. Five patients reported pregnancy after OC; 4 spontaneous and one using cryopreserved oocytes. The median and mean DRS scores were 10 (interquartile range: 25) and 13.4 (range 0-70), respectively, indicating a low prevalence of regret. Eighty-five (52.5%) women reported mild regret and 26 (16%) moderate to severe regret. Increased decision regret was associated with perception of inadequate emotional support during OC ($p=0.045$) and low expectations about the efficacy of the procedure($p=<0.001$). Decision regret was not associated with age, number of frozen eggs, perceived adequacy of information prior to OC, patient-estimated probability of achieving a live birth and use of banked eggs, attitude towards alternative child bearing methods, current relationship status or time elapsed since OC. One hundred sixty-seven women (88%) reported increased sense of control over future reproductive planning following OC. One hundred eighty-three women (89%) indicated they would be satisfied with their decision, even if they never used their frozen eggs.

Limitations, reasons for caution: One of the main limitations of our study is that these results reflect the attitudes of highly educated Turkish women with DOR towards OC, which should be extrapolated to other populations with caution. The other limitation is the relatively short and variable follow-up duration of the study.

Wider implications of the findings: Findings of this study indicate that the prevalence of regret among patients with DOR undergoing OC is low. Hence, the number of patients who return to use their oocytes is low, this restricts our ability to assess the impact of reproductive failure. Long-term studies including patients with failure are necessary.

Trial registration number: None

P-477 Assessment of the psycho-affective repercussions of male infertility

F. Hammami[1], A. Zouari[2], S. Ellouze[2], G. Rim[2], M. Chibani[3], R. Rachdi[3], S. Jellad[1]

[1]Military hospital of Tunis, Laboratory of Reproductive Biology- Unit of Assisted Medical Procreation, Tunis, Tunisia ; [2]Razi hospital, Department of Psychiatry B, Mannouba, Tunisia ; [3]Military hospital of Tunis, Unit of Assisted Medical Procreation Military Hospital of Tunis, Tunis, Tunisia

Study question: This study aims to evaluate the prevalence of anxiety and depressive disorders in men after diagnosis of their infertility.

Summary answer: Diagnosis of infertility is associated with important prevalence of anxiety and depressive disorders in men. Severity of these disorders increases with age and infertility's duration.

What is known already: Studies comparing women and men found that women react more strongly overall to infertility. The impact of psychological wellness in infertile women has been widely studied unlike the consequences of male psychological disorders that are not well explored. Similar to their female counter parts, men seeking fertility treatments have also an increased prevalence of anxiety and depression.

Study design, size, duration: A cross sectional study was conducted between June and September 2019 in the laboratory of reproductive Biology and Unit of assisted medical procreation of Military Hospital of Tunis.

Participants/materials, setting, methods: Data of 108 men consulting for couple's infertility exploration was included. All patients were interviewed on sociodemographic characteristics, medical and surgical history. We specified also the type and the duration of the infertility and we analyzed different semen parameters for each patient. Sperm abnormalities were classified as normal, moderate, severe, extreme and azoospermia. We used Hospital Anxiety and Depression Scale (HADS) to assess anxiety and depression.

Main results and the role of chance: The mean age of participants was 36,8 years. Eleven patients had a history of varicocele and six of them suffered from associated erectile dysfunction. Infertility was primary in most of patients (77,8%) with an average duration of 3,32 years. About 25% of the patients had at least one previous failed assisted reproductive attempt. Spermogram abnormalities were found in 78,7% of patients. 8,33% of them suffered from azoospermia. Depression was diagnosed in 29,7% of patients and anxious state in 34,2% of them. The severity of depression was correlated to anxiety ($P < 0,001$), older age of patients ($P=0,034$), and high infertility duration ($P=0,037$). However, the severity of sperm abnormalities was not correlated to anxiety or depression. No associations were found between psychological distress and erectile dysfunction or previous failed assisted reproductive technique attempt.

Limitations, reasons for caution: Diagnosis was established according to a self-reported scale without psychiatric consultation. Most of the couples had a feminine infertility factor, which might have generated a sample bias.
Wider implications of the findings: Anxiety and depression should be diagnosed and treated in men seeking for fertility treatment, especially that it can lead to accelerated cellular aging, poor quality gametes, and other complications. Systematic screening for these psychiatric disorders in infertile men population is therefore essential, in order to ensure multidisciplinary and adequate care.
Trial registration number: Not applicable

P-478 Transgender and Fertility preservation: how to assist young adults and adolescents transgender pathway

N. Mendes[1,2], A. Condat[2], J. Chambry[3], B. Bennanni Smires[1], S. Sarandi[1], L. Chobe Christian[1], L. Martinerie[4], F. Eustache[1]

[1]Service de Biologie de la Reproduction- CECOS- Hôpital Jean-Verdier- Avenue du 14 Juillet- 93140 Bondy- France, Fertility center, Bondy, France ;
[2]Service de Psychiatrie de l'Enfant et de l'Adolescent- Hôpital Pitié-Salpêtrière- 47-83 boulevard de l'Hôpital- 75013 Paris- France, Psychiatry, Paris, France ;
[3]Centre interhospitalier d'accueil permanent pour adolescents Paris rive droit- Hôpital Maison Blanche- Paris- 75018- France, Psychiatry, Paris, France ;
[4]Service d'Endocrinologie et de Diabétologie Pédiatrique- Hôpital Robert Debré & Université de Paris- Paris- 75019- France., Endocrinoly, Paris, France

Study question: Do our national counseling protocol and social insurance system improve access of fertility preservation (FP) prior hormonal therapy or sex reassignment surgery for transgender adolescents in comparison to international experience?
Summary answer: Two-thirds of the cases from our fertility center desire to FP. The obstacle in France seems to be the impossibility of using preserved gametes.
What is known already: International guidelines recommend that healthcare personnel should discuss FP with transgender individuals before starting gender affirmation therapy in adults and adolescents. Unfortunately, low access rates to FP center are reported. Many factors can affect the decision to undergo a FP, as laws, financial status, social climate and attitude of medical professionals ... Several studies indicate the risk of regret after a potential irreversible treatment. The nation social insurance system improve access to fertility preservation in France. It is important that transgender adolescents and adults be advised in the light of their vulnerability.
Study design, size, duration: From january 2018 to january 2020, 67 trans people (adults and adolescents), AMAB (assigned male at birth) (n=38) and AFAB (assigned female at birth) (n=29), were seen at our ART center.
Participants/materials, setting, methods: All trans people were seen first by a medical biologist and a psychologist to discuss and explain FP processes and the French law. In line with the psychiatrists and endocrinologists, a reflection period was proposed, then FP was performed whenever the person was willing to and organized in accordance with their choice. Comparison between adults and adolescents wish for fertility preservation and the method chosen were analyzed using student t test.
Main results and the role of chance: Mean age was 20.7 +/-6.7 years. Of all trans people, 75% were willing to undergo FP but only 42% actually went through the process. Almost 20% remained undecided. Among AMAB, 82% wished to undergo FP (mean age 23.4 +/-7.7), in comparison to 66% of AFAB (mean age 17 +/-1.8). No difference was found in the desire for FP between adults and adolescents (p=0.503). However, adults were more likely to actually go through the process of FP than adolescents (p<0.01). Qualitative data collected from psychological and biological evaluations suggested this result what related to the difficulty, consistent with the French Law, to reuse the preserved gametes after change of civil gender status.
Limitations, reasons for caution: These first results will need to be confirmed on a larger cohort.
Wider implications of the findings: Psychological counseling is in need of more data to assist trans people in the pathway decision. If financial aspect is not an argument to understand the difference between adults and adolescents fertility decision, is important to investigate further tracks.
Trial registration number: not applicable

P-479 Answer patterns by recurrent pregnancy loss patients on depression and stress scales

A. Slot[1], M.C. Krog[2], S. Bliddal[3], L.R. Olsen[4], H.S. Nielsen[5], A.M. Kolte[6]

[1]Copenhagen University Hospital- Rigshospitalet, The Recurrent Pregnancy Loss Unit- The Fertility Clinic, Copenhagen, Denmark ;
[2]Copenhagen University Hospital- Rigshospitalet, The Recurrent Pregnancy Loss Unit- The fertility Clinic and Department of Clinical Immunology, Copenhagen, Denmark ;
[3]Copenhagen University Hospital, Departments of Medical Endocrinology, Copenhagen, Denmark ;
[4]Mental Health Services in the Capital Region of Denmark, Child and Adolescent Mental Health Center, Copenhagen, Denmark ;
[5]University of Copenhagen- Rigshospitalet and Amager-Hvidovre Hospital, The Recurrent Pregnancy Loss Unit and Department of Gynaecology and obstetrics, Copenhagen, Denmark ;
[6]Copenhagen University Hospital, Recurrent Pregnancy Loss Unit- The Fertility Clinic, Copenhagen, Denmark

Study question: Are there specific patterns in how women with recurrent pregnancy loss (RPL) answered the Major Depression Inventory (MDI) and the Perceived stress scale (PSS)?
Summary answer: Women with RPL often feel guilt, angered and lack self-confidence. Women with primary RPL are a particularly sensitive group.
What is known already: RPL affects between 1-3% of all couples trying to conceive a child and the grief of experiencing pregnancy losses are to some women comparable to the grief of experiencing a neonatal death. Women with RPL more often suffer from psychological comorbidities such as anxiety, stress, and depression compared with other women trying to have a child. Recently, a qualitative study underlined the need for all involved health care professionals to be sensitive to the couples' history of pregnancy losses and to acknowledge their grief.
Study design, size, duration: This is a secondary analysis of a cohort study including women referred to the RPL Unit at the Fertility Clinic at Rigshospitalet in Denmark June 2010 -June 2013. In total, 302 women completed the MDI and the PSS. The prevalence of psychological stress and depression as summarized scores was published by our research group in 2015. Here we investigated if there were patterns in responses to the individual items on the MDI and the PSS.
Participants/materials, setting, methods: RPL was defined as ≥3 consecutive pregnancy losses, and 162 women had primary RPL (experienced RPL without having a child), 136 had secondary RPL (having a child prior to experiencing RPL), and four had tertiary RPL. The women's answers were reviewed and the cut-off for a "confirmative" answer was "slightly less than half the time" or more often on the MDI and "sometimes" or more often on the PSS.
Main results and the role of chance: The most frequent answers to the MDI were the three core symptoms of depression (42% lack of energy, 35% feeling sad, 35% loss of interest,) along with feeling less self-confident (28%) and guilty (26%). The cohort included 26 women who fulfilled the criteria for moderate to severe depression, 19 had primary RPL and 7 had secondary RPL. Of the 276 women who did not fulfill the criteria for moderate/severe depression, 9 women (3%) "felt that life wasn't worth living". The most frequent answers in PSS evaluation were being "angered because of things outside their control" (76%) and not feeling "things were going your way" (71%). Women with primary RPL answered that they felt less self-confident (32% vs 18%, p=0.007), restless (17% vs. 9% p=0.033), had concentration problems (25% vs. 10%, p=0,001) and that life wasn't worth living (13% vs. 3%, p=0.002) significantly more often compared with women with secondary RPL. In the PSS evaluation women with primary RPL more often reported "being unable to control important things" (69% vs 55%, p=0.018) and "unable to handle personal problems" (38% vs. 25%, p=0.020) compared with the women experiencing secondary RPL. There were no significant differences between the groups regarding the remaining items.
Limitations, reasons for caution: The questionnaires were constructed to result in a combined score or as a clinical tool for diagnosing, and not to analyse each item individually. Confirmatory answers on the MDI in the study presented here does not equal clinical depression but reflect prevalent feelings among women with RPL.
Wider implications of the findings: Women with RPL commonly experience guilt, lack of self-confidence, and being angered. Women with primary RPL

may be a particularly sensitive group. Treating clinicians need to understand the psychological impact of RPL and could try to encourage behavior to empower the women.

Trial registration number: Not Applicable

P-480 Understanding parents' intention to share information about the donor-conception with their offspring by application of the Theory of Planned Behavior

C. Lampic[1], A. Skoog Svanberg[2], K. Sorjonen[3], G. Sydsjö[4]

[1]Karolinska Institutet, Women's and Children's Health, Stockholm, Sweden ;
[2]Uppsala University, Women's and Children's Health, Uppsala, Sweden ;
[3]Karolinska Institutet, Clinical Neurosciences, Stockholm, Sweden ;
[4]Linköping University, Clinical and Experimental Medicine, Linköping, Sweden

Study question: Does the Theory of Planned Behavior contribute to understanding parents' intentions to share information about genetic origin with their child?

Summary answer: Parents' intention to start disclosure was influenced by beliefs that disclosure would have desired consequences and a desire to act in accordance to societal norms.

What is known already: There is growing consensus on donor-conceived persons' right to information about their genetic origin. As a result, there an increased use of identity-release donation and parents are encouraged to share information about the donor conception with their child from an early age. Nevertheless, disclosure to the child remains a challenge for many parents, particularly heterosexual couples, who are concerned about disrupted relationships and uncertain about when and how to talk with the child. The Theory of Planned Behavior (TPB) has been shown to explain various health-related behaviors and may be applied to increase the understanding of parents' decision-making regarding disclosure.

Study design, size, duration: The present study is part of the prospective longitudinal 'Swedish Study on Gamete Donation' (SSGD) including all fertility clinics performing gamete donation in Sweden. Consecutive groups of recipient couples were recruited 2005-2008 and followed with individual questionnaires before and after treatment with oocyte and sperm from identity-release donors. The present study includes data from the follow-up assessment of the SSGD when participants' offspring had reached age 7.

Participants/materials, setting, methods: Eligible were heterosexual recipients with 7-year old children following identity-release oocyte donation (OD, n=147) and sperm donation (SD, n=174). A total of 83 OD-parents (56% response) and 113 SD-parents (65% response) responded to a postal survey. They individually completed the study-specific TPB-Disclosure Questionnaire assessing behavioral, normative and control beliefs and the intention to talk with child about the donor conception during the upcoming year. Data were analyzed with Path analysis.

Main results and the role of chance: More than half of participants had already started talking with their 7-year old child about his/her donor conception (OD 61%, SD 58%). All components of the Theory of Planned Behavior significantly influenced parents' intention to talk with their child about his/her donor conception during the upcoming year. Among parents who had not yet started the disclosure process, their intention to talk with their child was influenced by beliefs that disclosure would have desired consequences and a desire to act in accordance to perceived positive attitudes towards disclosure in society. Interestingly, a higher level of control beliefs, i.e. feeling confident and in control over the disclosure process, was not significantly related to a stronger intention to talk with the child during the upcoming year. Type of treatment (OD/SD) and the existence/absence of a genetic link to the child did not directly influence parents' intention to talk with the child about the donor conception.

Limitations, reasons for caution: The study was performed with heterosexual couples within the context of the Swedish legislation that mandates identity-release donation, which limits the generalizability to other populations. Also, attrition may have introduced selection bias to the study findings.

Wider implications of the findings: The present results indicate that the Theory of Planned Behavior may contribute to understanding disclosure decisions among heterosexual couples building a family with donor gametes. Parents may benefit from opportunities to discuss perceived consequences of disclosure in order to support family life following donor conception.

Trial registration number: not applicable

P-481 Social aspects of infertility in Europe: a patient survey

M. Lopez-Teijon[1], M. Benigna[2], A. Garcia-Faura[1], B. Marques[1], S. Novo[1], F. Garcia[1], C. Carolina[1]

[1]Institut Marques, Reproductive Medicine Service, Barcelona, Spain ;
[2]Institut Marques, Reproductive Medicine Service, Roma, Italy

Study question: Do european patients have a feeling of social rejection to infertility? Has it changed during the last years?

Summary answer: 63,34% of patients believe that fertility treatments aren't accepted by the society in which they live, and their feeling of rejection by society is increasing.

What is known already: Each country offers a different cultural context in which to view infertility, and legislation controlling fertility treatments also forms opinion. In Europe the law differs very widely between countries, with some countries being extremely restrictive (such as Germany) and others, such as Great Britain or Spain, where infertility treatments are far more visible in society.

Study design, size, duration: Between June-December 2019 questionnaires were filled in by 1733 patients undergoing fertility treatment, from 10 different countries. We asked 3 questions:

Do you think that in your country fertility treatments are taboo?

Have you told the people in your immediate environment that you are undergoing fertility treatment?

Would you agree to be interviewed in the media in order to help other people?

We compared the results to those obtained from the same questionnaire in 2013.

Participants/materials, setting, methods: The questionnaire was completed by patients currently in treatment at our clinics in Spain, Italy and Ireland. They were completed by female patients (either single, with a male partner or with a female partner). We performed a descriptive analysis of the results.

Main results and the role of chance: For 63,3% of patients fertility treatment is a taboo subject. In 2013, this percentage was 52,3%, indicating that infertility as a taboo subject increased in nearly all countries.

However, the social perception of infertility within Europe was very variable. The greatest feeling of rejection was found in Italy (74%) and Germany (74%). Ireland (56%), Switzerland (55%), France (54%), Spain (52%), Sweden(50%) and UK(47%) were in the middle. Belgium was the most tolerant (36%).

It is interesting to note that the most tolerant countries in 2013 no longer are so; Spain increased by 17% and the UK by 11%.

87% of patients did share their experience of infertility with their closest family and friends but without giving any details of the treatment. This was 12% more than in 2013. The family continues to provide a point of refuge but now 37% more patients inform everyone in their immediate environment of their treatment.

With regard to being willing to discuss their infertility with the media, 68% would now agree to do so (in 2013 55% agreed). Maintaining their anonymity is still an indispensable factor for the majority of patients.

There is very little variability in reponses between patients from different countries for the last two questions.

Limitations, reasons for caution: The nature of the questions and the inclusion of personal values regarding the concept of a 'taboo' issue could give rise to ambiguity and therefore limit the validity of some replies to the questionnaire.

Wider implications of the findings: The results allow us to better define the psychological needs of infertile patients, and to see how the context of their country of origin may affect them.

It's surprising that the increasing success of techniques in reproductive medicine isn't accompanied by a similar increase in social acceptance of the treatments.

Trial registration number: Not applicable

P-482 Characteristics and intentions of heterosexual couples comprised of a transgender man and his cisgender woman awaiting sperm donation to conceive a child.

S. Bonan Benchetrit[1], N. Mendes[2], E. Chapel-Lardic[1], O. Rosenblum[2], C. Dudckiewicz-Sibony[1], J.P. Wolf[1], A. Condat[2], C. Chalas[1], C. Patrat[1], V. Drouineaud[1]

[1]Assistance Publique – Hôpitaux de Paris AP- HP Centre- Université de Paris-, Service de Biologie de la Reproduction - CECOS-, Paris, France ;

[2]Assistance Publique-Hôpitaux de Paris AP-HP- Groupe Hospitalier de La Pitié-Salpétrière-, Service de Psychiatrie de l'enfant et de l'adolescent-, Paris, France

Study question: What are the main characteristics and intentions of couples comprised of a transgender man and his cisgender woman awaiting sperm donation?
Summary answer: The couples often met before the man's transition, shared a common life of several years, intend to inform their child of sperm donation and transidentity.
What is known already: It has been reported that more than half of transgender men (female-to-male transgender persons) desired to have children. Since 1994, Assisted Reproductive Technologies (ART) are strictly offered to heterosexual couples in France. Indeed, in order for a transgender man to have access to ART to conceive a child, he must be in a relationship with a woman and have changed his legal sexual identity from female to male. Very little data have been previously reported about the characteristics and intentions of heterosexual couples in which the man is transgender and awaiting sperm donation to conceive.
Study design, size, duration: A retrospective analysis of unit records of 43 heterosexual couples comprised of a transgender man with his cisgender female partner, who apply for ART with donor sperm to conceive a child, was performed. Couples were recruited at the Centre de Conservation des Oeufs et du Sperme (CECOS) (APHP, Cochin, Paris) between October 2010 and December 2019. Couples were asked questions of qualitative and quantitative nature.
Participants/materials, setting, methods: The study was set in the CECOS of (APHP, Cochin, Paris). Our study reported here sought to analyze: socio-demographic characteristics of the couples, personal history of the transgender man, sex transition, meeting and life of the couple, intentions regarding disclosure of sperm donation and father's transidentity to the child.
Main results and the role of chance: Men and women were 32±6.6 and 29.7±4.6 years old, respectively. 37% of transgender men had a higher level than the bachelor degree. Most men (65%) and women (67%) were employees. Transgender men often reported first manifestations during early childhood (54%), difficult experience of breast development (70%) and menstruations (79%). 40% of men reported difficulties during the school and an unstable professional career. Sex transition process was often triggered by a television program (28%) or following web/social networks use (28%). Importantly, in 77% of the cases, the couple met before the man's transition. Most women were heterosexual (60.5%), some identified as lesbian (2.3%) [CP1] or bisexual (14%). 81% of women were comprehensive at the announcement of transidentity. The couples were often stable with a good socio-professional integration and a common life of at least 3 years in 80% of the cases. 4.6% of men and 11.6% of women previously have had a child with another partner. 7% of couples had already a child conceived by sperm donation and 54% had a child project for less than 2 years and 41% for 3 to 5 years. Almost all couples intended to inform their child of sperm donation (95%) and father's transidentity (90%).
Limitations, reasons for caution: The profile of our couples probably does not reflect that of all couples consisting in a transgender man and a cisgender woman. The study spends a long period and couples' characteristics could probably change over the time. The French legislation is still restrictive and influences the profile of those couples
Wider implications of the findings: The future Bioethics law should allow the access to ART for single women and lesbian couples and will probably allow the profile of couples composed of a transgender person to be diversified.
Trial registration number: NA

POSTER VIEWING SESSION

REPRODUCTIVE (EPI)GENETICS

P-483 Embryo selection after PGT-A. Day 5 embryos should be preferred to day 6, even if worst by morphology and time-lapse.

V. Nagornyy[1]

[1]Clinic of Reproductive Medicine 'NADIYA', Embryology department, Kiev, Ukraine

Study question: Should a good morphology day 6 embryo be chosen over day 5 poor morphology in a limited options scenario of PGT-A embryo transfer?
Summary answer: Day 6 PGT-A embryos produced less pregnancies in our settings even when compared to the day 5 "BB grade only" embryo transfers.
What is known already: To date, many studies have shown that day 6 embryos seem to have a reduced potential for achieving pregnancy when compared to day 5 embryos. However, most of those included data of non-PGT-A embryos, while day 5 embryos are well known for having higher euploidy rates. If PGT-A embryos were compared, authors usually were unable to conclude any differences in pregnancy rates between euploid day 5 and day 6 embryos. So, during the laboratory routine there is no obvious choice if only good morphology day 6 and impaired morphology day 5 embryo are available for PGT-A transfer.
Study design, size, duration: This was the retrospective analysis of 1331 PGT-A single embryo transfers in regard to embyo culture day, morhphological score and pregnancy outcome. When available the time-lapse analysis data were included in the comparison.
Participants/materials, setting, methods: PGT-A analysis was performed by aCGH method in
Main results and the role of chance: From 1331 PGT-A single embryo transfers the day 6 embryos were used in 342 cases (25.7%), day 5 "BB grade only" in 101 cases (7.6%). Surprisingly, corresponding clinical pregnancy rates were 45.0% and 54.5%.

When morphology of embryos transferred after biopsy at day 6 was analysed, we observed that 41.6% of those had AA grade, 31.1% were AB or BA and only 28.3% were BB scored. Thus, this group had obviously better scores than day 5 "BB grade only" cohort and difference was not caused by poor morphology.

After we compared the distribution of embryo transfer orders for day 6 AA and day 5 "BB grade only" transfer, we can conclude that in most cases the reason for day 6 embryo selection was not the fact that all day 5 euploid embryos were already transferred in the previous attempts. In fact, the day 5 "BB grade only" transfers occurred after several unsuccessful transfers more often.Time-lapse data indicates that day 6 embryos had slower development starting at 6 cell stage with increasing lag onward, otherwise performing similar.

This data leads us to conclusion that day 6 embryos produce less pregnancies due to their intrinsic values other than morphology and chromosomal constitution.
Limitations, reasons for caution: This is a retrospective analysis and thus it may be subject of bias.
Wider implications of the findings: Our data seem to suggest that it may be better to avoid culture of embryos until day 6 if biopsy is possible at day 5 .
Trial registration number: Not applicable

P-484 FMR1 and AKT/mTOR signaling in human granulosa cells: functional interaction and impact on ovarian response

J. Rehnitz[1], E. Capp[2], B. Messmer[1], A. Germeyer[3], J.E. Dietrich[3], T. Strowitzki[3], P.H. Vogt[1]

[1]University Women´s Hospital, Gynecological Endocrinology and Fertily Disorders - Division of Reproductive Genetics, Heidelberg, Germany ;
[2]Universidade Federal do Rio Grande do Sul, Department of Obstetrics and Gynecology- Medicine School, Porto Alegre, Brazil ;
[3]University Women´s Hospital, Gynecological Endocrinology and Fertily Disorders, Heidelberg, Germany

Study question: Does *FMR1* impact AKT/mTOR-signaling-pathway in human granulosa cells (GC) and is this relevant for ovarian response?
Summary answer: *FMR1*-expression significantly correlates with *AKT-, TSC2-, mTOR-* and *S6K*-expression in GC of women with different ovarian-response indicating a functional effect of *FMR1*/FMRP on AKT/mTOR-regulated follicular-maturation.
What is known already: *FMR1* controls folliculogenesis and human oocyte-maturation. Its transcript and protein (FMRP) is highly expressed in women´s GC. Increased *FMR1* expression levels lead to reduced FMRP production (i.e. negative feed-back loop) causing premature ovarian insufficiency/failure (POI/POF) in 20% of cases.
FMRP is supposed to control translation of *Tsc2* and *mTor*, both members of the AKT/mTOR signaling-pathway, via RISC-(RNA-induced-silencing-complex)-formation.

AKT/mTOR-signaling controls primordial follicle activation and therewith ovarian-reserve. *FMR1*/FMRP expression and mTOR/AKT-signaling are putatively linked in human GCs as their expression was altered in cell culture after FSH-stimulation or inhibition of AKT with MK-2206-2HCl or mTOR with rapamycin, respectively.

Study design, size, duration: 297 fertile women undergoing controlled ovarian stimulation for IVF/ICSI-treatment were recruited from 2013 to 2019 in our outpatient clinic in Heidelberg. They were divided according to their ovarian response into either NOR: normal (n: 217) or POR: poor responders (n: 80) (according to ESHRE guidelines).This study was approved by the local ethical committee and conducted according to the principles of the Declaration of Helsinki.

Participants/materials, setting, methods: All patients provided written informed consent and completed a clinical questionnaire.

mRNA was extracted from GCs after follicular aspiration and oocyte separation; quantitative expression analyses of *FMR1, AKT, TSC2, mTOR, S6K, FOXO3, FOXO1* genes were performed with specific TaqMan-Assays.

Statistical analyses with SPSS; significance set $p<0.05$.

Main results and the role of chance: Rate of *FMR1* expression significantly correlates with *AKT-, TSC2-, mTOR-, S6K*-expression levels ($p< 0.001$ for all; correlation coefficients (cc): 0.515 for *AKT*, 0.458 for *TSC2*, 0.542 for *mTOR* and 0.465 for *S6K*) suggesting a functional linkage. In patients with POR those effects were even more prominent with the biggest increase for *mTOR* and *S6K* (cc: 0.763 for *mTOR*; 0.656 for *S6K;* all $p<0.001$).

Functional interaction between FMRP and transcripts of these members of the AKT/mTOR signal pathway seem therefore to be required for follicular oocyte maturation.

Interestingly, corresponding expression analyses of some downstream members of this signal pathway, *FOXO3* and *FOXO1*, in NOR and POR samples did not display association with *FMR1* expression levels. But they demonstrated higher expression of *FOXO1* in NOR and lower in POR, associated each with inverse *AKT*-levels. This suggests a negative feedback mechanism for these genes also with a putative impact on women´s ovarian reserve.

Limitations, reasons for caution: Control of human folliculogenesis and oocyte maturation by AKT/mTOR-signal-pathway is well known. To prove proposed functional interactions to the molecular control of *FMR1*/FMRP-expression in GC significant numbers of NOR- and POR-samples in different age groups have to be collected to exclude age dependent expression variabilities of these genes.

Wider implications of the findings: Results confirm our earlier expression analyses performed in granulosa cell cultures (COV434) pointing already to a linkage of *FMR1*/FMRP expression with the mTOR/AKT-signaling pathway.

Results will also help to improve POR-diagnostic in women before controlled stimulation for subsequent IVF/ICSI-procedures to raise their chances for sufficient mature follicles.

Trial registration number: not applicable

P-485 Noninvasive preimplantation genetic test for aneuploidy (NIPGT-A) has a lower false positive rate than that of the invasive PGT-A

L. Vagnini[1], C.G. Petersen[1,2], A. Renzi[1], J.B.A. Oliveira[1,2], A.H. Oliani[3], R. Nakano[4], C.G. Almodin[5], C. Marcondes[6], A. Ceschin[7], A. Amaral[8], E. Borges Jr[9], A. Castelo Branco[10], J.B. Soares[11], J. Lopes[12], J.G. Franco Jr.[1,2]

[1]*Paulista Center for Diagnosis- Research and Training, Research, Ribeirao Preto, Brazil ;*
[2]*Centre for Human Reproduction Prof. Franco Jr, Research, Ribeirao Preto, Brazil ;*
[3]*Sao Jose do Rio Preto School of Medicine FAMERP, Research, Sao Jose do Rio Preto, Brazil ;*
[4]*Ferticlin Human Fertility Clinic, Research, Sao Paulo, Brazil ;*
[5]*Materbaby, Research, Maringa, Brazil ;*
[6]*Santista Nucleus of Human Reproduction, Research, Santos, Brazil ;*
[7]*Feliccita Fertility Institute, Research, Curitiba, Brazil ;*
[8]*Genesis Human Reproduction Assistance Center, Research, Brasilia, Brazil ;*
[9]*Fertility Medical Group, Research, Sao Paulo, Brazil ;*
[10]*Art Fertil Human Reproduction Clinic, Research, Recife, Brazil ;*
[11]*Alpha Project - Alliance of Assisted Fertilization Laboratories, Research, Sao Paulo, Brazil ;*
[12]*CENAFERT, Research, Salvador, Brazil*

Study question: Does NIPGT-A have lower false positive rates (FPR) than invasive PGT-A?

Summary answer: When DNA sequencing from whole embryo cells was used as the gold-standard, the FPR of NIPGT-A was 3.32-times smaller than that obtained with invasive PGT-A.

What is known already: After many years of using PGT-A, there are still many concerns, such as risks of invasive action and difficulties in the correct interpretation of mosaicism, which could lead to errors in the interpretation of false positive and negative results. Recently, a new technology (NIPGT-A) has arisen using cell-free DNA present in the spent culture media of human blastocysts. Unlike invasive PGT-A that uses only trophoblastic cells, NIPGT-A reflects the ploidy status of trophoblastic cells and inner cell mass, suggesting that this new technology could be less prone to errors, being more reliable than invasive test.

Study design, size, duration: This cohort study included a total of 37 blastocysts vitrified on day 5 that were previously biopsied for invasive PGT-A and presented a diagnosis of aneuploidy. The embryos were donated under informed consent by patients following the Human Medical Authority regulations. Blastocysts were thawed and cultured in 15µl drops of culture medium under oil. After their expansion (4-8hours), the blastocysts were transferred to NGS tubes and their corresponding spent media were collected for analysis.

Participants/materials, setting, methods: The DNA of all samples (spent culture medium and whole embryo) was amplified by the MALBAC® technology (Yikon Genomics). The samples were subjected to next-generation sequencing (NGS) using Illumina MiSeq® System. The ploidy status results obtained from ChromGo™ software (Yikon Genomics) for culture medium and whole embryo were compared to determine the accuracy of NIPGT-A for screening chromosomal abnormalities in each embryo.

Main results and the role of chance: DNA from all 37 spent media samples and whole embryos were successfully amplified. Comparing the results of NIPGT-A and whole embryos sequencing, the positive predictive value (PPV) was 93.5% and the FPR was 6.5%. On the other hand, comparing the whole embryo and invasive PGT-A results, the PPV was 78.4%, and the FPR was 21.6% (Table 1). Both NIPGT-A and invasive PGT-A had a negative predictive value (NPV) of 100% and a false negative rate (FNR) of 0%. In the eight cases of disagreement the results are presented in the Table 2.

Table 1. NIPGT-A and Invasive PGT-A results

NIPGT-A	Whole Embryo		Invasive PGT-A	Whole Embryo	
	Aneuploidy	Normal		Aneuploidy	Normal
Aneuploidy	29	2	Aneuploidy	29	8
Normal	0	6	Normal	–	–

PPV: 93.5% FPR: 6.5%
PPV: 78.4% FPR: 21.6%

Table 2. Disagreement results of whole embryo, NIPGT-A and invasive PGT-A

Whole embryo	NIPGT-A	Invasive PGT-A
46,XY	46,XY	XY,+1q(x3);+3q(x3)
46,XY	46,XY	XY,-2(x1)
46,XY	XY,-1(x1);-9q(x1)	XY,+9q(x3)
46,XX	46,XX	XX,+9q(x3)
46,XX	46,XX	XX,-4(x1)
46,XY	46,XY	X0, multiple abnormalities
46,XX	46,XX	XX,+13(x3)
46,XY	XY,-1(x1);-9(x1),-19(x1);-21(x1)	XY,-9(x1)

Limitations, reasons for caution: The sample size was relatively small, however comparative analysis between the results of invasive and noninvasive PGT-A with whole embryo are rare. All donated embryos were classified as aneuploidy by invasive PGT-A. Additionally, the cut-off for aneuploidy in cases of invasive PGT-A could be variable (multicenter study).
Wider implications of the findings: NIPGT-A has a lower FPR than invasive PGT-A and does not require micromanipulation skills, avoiding trophectoderm biopsies trauma and seems to provide more accurate results corresponding to the ploidy status of the whole embryo. Thereby NIPGT-A should be considered as the test of choice for genetic evaluation of the embryo.
Trial registration number: Not Applicable

P-486 Does day of PGT-A biopsy predict mosaicism rates?

B. McGuinness[1], B. Abittan[2], A. Pratt[3], W. Shan[4], S. Brownridge[2], R. Goldman[2], M. Christine[2]

[1]NYU Winthrop Hospital, Obstetrics and Gynecology, Mineola, U.S.A. ;
[2]Northwell Health Fertility, Reproductive Endocrinology and Infertility, Manhasset, U.S.A. ;
[3]Sanford H. Calhoun High School, General, Merrick, U.S.A. ;
[4]The Feinstein Institute for Medical Research, Biostats Unit, Manhasset, U.S.A.

Study question: Does the rate of mosaicism differ between blastocysts biopsied on day 5 (D5) versus day 6 (D6) using NGS (Next-Generation Sequencing) technology for PGT-A (Preimplantation Genetic Testing for Aneuploidies)?
Summary answer: Although rate of aneuploidy is higher with embryos biopsied on D6 versus D5, rate of detected mosaicism does not vary based on blastocyst biopsy day.
What is known already: Advancing technologies in the field of assisted reproductive technology has sensitized our methodologies with the utilization of NGS in identifying trophectoderm (TE) mosaicism (≥ 2 distinct cell lines within a TE biopsy). The timing of post-fertilization mitotic errors affects the percentage of abnormal cells present within a mosaic embryo, but it is not known if the rate of mosaicism is related to the day of TE biopsy.
Study design, size, duration: This is a retrospective cohort study of patients who underwent IVF/PGT-A using NGS technique from January 2018 to July 2019. A total of 663 patients underwent 797 IVF/PGT-A cycles. 50 cycles were excluded due to incomplete data for analysis.
Participants/materials, setting, methods: The numbers of euploid, aneuploid, low and high-level mosaic embryos were collected. Of the included cycles, 257 cycles (34.4%) had embryos biopsied on both day 5 (D5) and day 6 (D6). These cycles were analyzed using paired non-parametric testing to compare the D5 and D6 rates of mosaicism. The Wilcoxon rank-sum was used to analyze rates of euploidy, aneuploidy, total mosaicism, low-level mosaicism, and high-level mosaicism. Significance was defined by $p < 0.05$.
Main results and the role of chance: The rates of total mosaicism (15.7% vs. 14.2%; p=0.19), low-level mosaicism (61.5% vs. 58.9%; p=0.14), and high-level mosaicism (38.5% vs. 41.1%; p=0.09) did not differ significantly between D5 and D6 biopsies respectively. Embryos biopsied on D5 were less likely to be aneuploid and more likely to be euploid than those biopsied on D6 (Aneuploid: 33.3% vs. 44.2%; p=<0.001. Euploid: 48.8% vs. 38.8%; p=<0.001). Comparison of matched samples determined that D5 and D6 blastocytes have the same distribution of mosaicism and do not have the same distribution of aneuploidy or euploidy.
Limitations, reasons for caution: This study is limited by small sample size. 797 IVF/PGT-A cycles occurred but only 257 cycles had embryos biopsied on both D5 and D6 and complete data for analysis. Further research is needed to better understand the in vitro development of mosaic embryos and the underlying mechanisms of blastocyst formation.
Wider implications of the findings: Although studies have examined the detection of blastocyst stage mosaicism with use of PGT, no study has used paired non-parametric testing to compare D5 and D6 rates of mosaicism as was done here. This study supports the argument that timing of PGT biopsy does not affect rate of detected mosaicism.
Trial registration number: not applicable

P-487 The polymorphism Ala307Thr of the Follicle Stimulating Hormone Receptor (FSHR) gene is associated with different doses of recombinant FSH received during IVF/ICSI treatment.

F. Dieamant[1,2], C.G. Petersen[1,2], L.D. Vagnini[2], A. Renzi[2], M. Canas[2], B. Petersen[1,2], A.L. Mauri[1,2], M. Mattila[1], J. Ricci[1], A. Nicoletti[1], C. Zamara[1], A.H. Oliani[3], J.B.A. Oliveira[1,2], J.G. Franco Jr.[1,2]

[1]Centre for Human Reproduction Prof Franco Jr, Research, Ribeirao Preto, Brazil ;
[2]Paulista Center for Diagnosis- Research and Training, Research, Ribeirao Preto, Brazil ;
[3]Sao Jose do Rio Preto School of Medicine FAMERP, Research, Sao Jose do Rio Preto, Brazil

Study question: Is there an association between FSHR gene Ala307Thr polymorphism (rs6165) and ovarian reserve, ovarian response or clinical results in IVF/ICSI treatment?
Summary answer: Ala/Ala genotype was associated with the use of higher doses of recombinant FSH(r-FSH), suggesting that homozygosis of this allelic variant(Ala) provides lower sensitivity to r-FSH.
What is known already: Follicle-stimulating hormone (FSH) is essential for folliculogenesis and acts through the FSHR that is present on the membrane of granulosa cells. Polymorphisms in the FSHR gene may lead to an altered pattern of receptor expression on the cell surface or to changes in affinity for FSH. The Ala307Thr polymorphism is located in the extracellular domain within the hormone binding region, which can influence the response to endogenous and exogenous FSH stimulation. However, the influence of the FSHR Ala307Thr polymorphism on ovarian function is still controversial.
Study design, size, duration: This prospective cohort study included 450 women who underwent IVF/ICSI cycles. The enrolled individuals met the following inclusion criteria: age of ≤37 years; normal karyotype; presence of two ovaries as observed by ultrasound examination; and no history of ovarian surgery, endometriosis, hydrosalpinx, infection, or endocrine disorders.
Participants/materials, setting, methods: DNA was extracted from peripheral blood, and the Ala307Thr FSHR polymorphism (rs6165) was genotyped using TaqMan SNP genotyping assay. The results were associated with age, anti-Müllerian hormone (AMH) levels, antral follicle counts (AFC), total dose of r-FSH, follicle size, number of retrieved oocytes, and clinical outcome of IVF/ICSI cycles. The statistical analyses were performed using Fisher's exact test and Kruskal-Wallis test.
Main results and the role of chance: An association between the genotypes of the FSHR (Ala307Thr) polymorphism and different doses of r-FSH was observed. Patients with the Ala/Ala genotype received a higher r-FSH dose than patients with the Ala/Thr (P=0.0002) and Thr/Thr (P=0.02) genotypes. No other correlation was observed. Table 1 summarizes the results.
Limitations, reasons for caution: Possible limitation is the cross-sectional nature of the data. Differences in the genetic backgrounds of various ethnic populations might also be considered.
Wider implications of the findings: The results suggest that FSHR Ala307Thr (rs6165) gene polymorphism is related to ovarian response but not to ovarian reserve. This SNP can be used as an additional tool in the individualization of ovarian stimulation protocols.
Trial registration number: Not Aplicable

P-488 The live birth rate and the position of breakpoints in patients with recurrent miscarriage caused by reciprocal translocation

T. Sato[1], F. Ozawa[1], C. Saito[1], H. Yosihara[1], C. Banno[1], Y. Sawada[1], Y. Matsumoto[1], M. Sugiura-Ogasawara[1]

[1]Nagoya City University Graduate School of Medical Sciences, Department of Obstetrics and Gynecology, Nagoya, Japan

Study question: Can we estimate the frequency of alternate segregation from the position of breakpoints in patients with recurrent miscarriage (RM) caused by reciprocal translocation?
Summary answer: We could find no association between the frequency of alternate segregation and the position of breakpoints.

Table 1. Results

	FSHR (rs6165) genotypes			
	Thr/Thr	Thr/Ala	Ala/Ala	P
n (%)	141 (31.3%)	213 (47.4%)	96 (21.3%)	
Age	35.0±3.8	34.5±4.1	34.5±4.7	0.57
BMI (kg/m^2)	24.3±4.0	24.3±4.0	24.4±4.7	0.81
AMH (ng/ml)	1.7±2.3	1.9±2.1	1.6±2.1	0.26
AFC (n)	12.6±8.7	13.4±7.4	12.3±9.0	0.11
Total dose FSH (UI)	2085±922[a]	1946±955[b]	2364±1060[a,b]	[a]0.02; [b]0.0002
Time of stimulation (days)	10.3±2.5	10.1±2.4	10.5±2.2	0.17
Follicles (n): Total	11.4±6.6	12.0±7.4	11.6±7.4	0.86
Follicles (n): ≥18 mm	3.8±2.2	5.7±2.0	3.6±2.5	0.64
Retrieved oocytes: Total (n)	8.2±5.4	8.1±5.1	8.2±5.4	0.98
Retrieved oocytes Metaphase II (n)	6.1±4.3	5.9±4.1	6.1±4.8	0.92
Fertilization rate	64.5±26.4%	64.6±26.5%	64.4±29.9%	0.96
Implantation rate	23.9%	26.1%	25.7%	0.82
Pregnancy rate/transfer	34.6%	39.9%	39.8%	0.65
Pregnancy rate/patient	31.2%	35.2%	34.4%	0.79

What is known already: Preimplantation genetic testing for structural rearrangements (PGT-SR) is performed to prevent miscarriage worldwide, but it cannot improve the live birth rate in patients with RM. In carriers of reciprocal translocations, it has been reported that the proportion of each meiotic segregation mode depends on certain characteristics, including the gender of the carrier, the position of the breakpoints and the involvement of acrocentric chromosomes. However, there is no method for using the karyotype to estimate the frequency of alternate segregation ending in a live birth.
Study design, size, duration: This is a retrospective study to evaluate the proportion of meiotic segregation modes in PGT-SR performed at our hospital between 2007 and 2019. A total of 469 day 3 or day 5 embryos from 67 cycles of PGT-SR for 20 couples with RM caused by reciprocal translocation were examined by fluorescence in situ hybridization (FISH) or array comparative genomic hybridization (aCGH).
Participants/materials, setting, methods: For PGT-SR, FISH or aCGH was performed on biopsied samples of day 3 or day 5 embryos. According to the results, the meiotic segregation mode was classified, and the effects of the gender and age of the carriers, the size of the translocated segments, the ratio of the translocated segment to the size of the arm with the breakpoint and the form of the tetravalent chromosome structure in meiosis were evaluated.
Main results and the role of chance: Overall, 26% of 391 embryos with results were diagnosed with alternate segregation, 18% with adjacent-1 segregation, 8% with adjacent-2 segregation, 37% with 3:1 segregation, 2% with 4:0 segregation and 9% remained undiagnosed. There was no significant difference in the alternate segregation frequencies between 10 patients with more than 10 embryos with the meiotic segregation mode results. The frequency of alternate segregation was identical between male and female translocation carriers (26.1 vs 26.1%), while the incidence of the 3:1 segregation was significantly higher in female carriers than in male carriers (33.7 vs 19.1%, $p<0.05$). The age of the carrier did not affect the proportion of the meiotic segregation mode.No correlation was observed between the size of the translocated segments or its ratio to the size of the arm with the breakpoint and the frequency of alternate segregation.The frequency of adjacent-1 segregation was significantly higher in patients in which the sizes of both translocated segments were less than 50% of the size of each arm with the breakpoint compared with others (27.1 vs 12.1%, $p<0.01$).The form of the tetravalent chromosome structure in meiosis did not correlate with the frequency of alternate segregation.
Limitations, reasons for caution: The findings of the present study were limited by the relatively small sample size. In particular, more samples will be needed to evaluate the relationship between the form of the tetravalent chromosome structure in meiosis and the frequency of alternate segregation.
Wider implications of the findings: It might be possible to estimate the miscarriage rate because the frequency of adjacent-1 segregation which tends to end in miscarriage could be speculated from the position of the breakpoints. This information may be useful in the genetic counseling of couples who are reciprocal translocation carriers before performing PGT-SR.
Trial registration number: Not applicable

P-489 Is there a role for mitochondrial DNA quantification as a biomarker to select euploid blastocysts with high implantation potential?

V.C. Moreno[1], R.C.S. Figueira[1], S. Verza Jr[1], S.C. Esteves[2]

[1]Androfert – Andrology and Human Reproduction Clinic, Laboratory, Campinas/SP, Brazil ;
[2]Androfert – Andrology and Human Reproduction Clinic, Clinical Board, Campinas/SP, Brazil

Study question: Is mitochondrial DNA (mtDNA) quantification in trophectoderm cells a useful biomarker to select euploid blastocysts with a high implantation potential?
Summary answer: Although low mtDNA levels are associated with higher implantation rates, blastocyst morphology is more informative than mtDNA quantification to identify euploid embryos that implant.
What is known already: Transfer of euploid blastocysts does not invariably result in ongoing pregnancies, thus indicating that other factors impact embryonic implantation potential. Factors such as metabolic rates and adenosine triphosphate content have been shown to vary significantly in oocytes and embryos and could affect embryo viability. On this basis, embryo function, indirectly measured by mtDNA copy number, emerged as a potential quantitative biomarker for embryonic selection prior to transfer. Initial data have shown that euploid blastocysts with elevated mtDNA copy numbers rarely result in successful pregnancies. However, subsequent research could not confirm these observations and the literature remains equivocal.

Study design, size, duration: A prospective observational study was performed between September 2017 and September 2019 to analyze the contribution of mtDNA levels for embryo implantation in 160 euploid blastocysts obtained from 123 infertile couples undergoing preimplantation genetic testing for aneuploidy (PGT-A). Implantation outcomes were assessed based on mtDNA levels and blastocyst morphology criteria to determine the usefulness of these biomarkers to predict successful implantation in a single euploid blastocyst transfer program.
Participants/materials, setting, methods: Trophectoderm biopsies were subjected to whole genome amplification followed by comprehensive chromosome analysis via next-generation sequencing (NGS). mtDNA quantification was also performed using NGS. A mitochondrial score (Low - High) using the mitochondrial-to-nuclear DNA ratio (mt/gDNA) was applied. Statistical analyses included comparative analyses, correlation coefficients, and generalized linear models. Areas under the ROC curve (AUC) were estimated to assess the ability of the mt/gDNA and blastocyst morphology to predict implantation outcomes.
Main results and the role of chance: Implantation rates were significantly higher in Low-score (n=140) than High-score (n=20) euploid blastocysts selected for transfer (48.6% vs. 25.0%, p=0.048). Correlational analysis showed a significant but weakly positive correlation between mt/gDNA and successful implantation (Spearman's correlation, r=0.157, p=0.04). By contrast, a strong negative correlation was observed between blastocyst morphology and implantation occurrence (Spearman's correlation, r=-0.283, p < 0.0001). The logistic regression analyses showed no association between mt/gDNA and implantation, whereas blastocyst morphology had a positive influence on the likelihood of euploid blastocyst implantation (OR=5.103, 95% IC: 1.027-25.357, p=0.04). ROC analyses suggested that blastocyst morphology alone was able to better rank the embryos with positive implantation outcomes than mt/gDNA alone (AUC = 0.72635, p=0.05). The combination of both parameters conferred a small increase in the implantation predictive ability (AUC= 0.74725, p=0.04).
Limitations, reasons for caution: Our findings are based on a relatively small dataset. Another limitation is the lack of randomization as embryo morphology was the primary criterion for selecting euploid blastocysts for single embryo transfer. Thus, there was a difference in the number of embryos among the mt/gDNA score groups.
Wider implications of the findings: Although an association exists between mtDNA levels and implantation rates, mtDNA cannot be used as an independent biomarker to identify euploid blastocysts that result in successful implantation. Our findings suggest that at present blastocyst morphology should be prioritized in the clinical settings to rank euploid blastocysts with high implantation potential.
Trial registration number: None

P-490 The mean euploidy rate per cohort of biopsied blastocysts is independent of couples' previous reproductive history: an analysis of 2676 cycles with preimplantation-genetic-testing-for-aneuploidies.

D. Cimadomo[1], A. Capalbo[2], A. Vaiarelli[1], S. Colamaria[1], R. Maggiulli[1], L. Albricci[1], L. Dovere[1], M. Stoppa[1], L. Tacconi[1], F. Sanges[1], D. Soscia[1], A. Giancani[1], E. Scepi[1], F.M. Ubaldi[1], L. Rienzi[1]

[1]Clinica Valle Giulia, G.EN.E.R.A. centers for reproductive medicine, Roma, Italy ; [2]Igenomix, Igenomix Italy, Marostica, Italy

Study question: Is the mean euploidy rate per cohort of biopsied blastocysts (m-ER) associated with the previous reproductive history of the couples undergoing preimplantation-genetic-testing-for-aneuploidies (PGT-A)?
Summary answer: If adjusted for maternal age, couples' previous reproductive history has no effect on the m-ER after PGT-A.
What is known already: Several studies investigated the association between the euploidy rates after PGT-A and i) patients' characteristics (e.g. maternal/paternal age, sperm factor), ii) ovarian stimulation strategy (e.g. protocol, dose of gonadotrophins, trigger of ovulation), iii) IVF cycle characteristics (e.g. incubator, culture strategy), iv) blastocysts' features (e.g. morphological quality, developmental rate). Conversely, the expected m-ER after PGT-A due to couples' previous reproductive history (previous cycles, miscarriages, live births and lack of euploid blastocysts identified during prior cycles) still needs to be outlined. Yet, this information is critical to counsel the patients towards a first PGT-A cycle, or after former adverse outcomes.
Study design, size, duration: Observational study including all trophectoderm biopsy-based PGT-A cycles (n=2236 couples;2676 treatments;8151 embryos) conducted via comprehensive-chromosome-testing techniques (CCT: qPCR or NGS;Igenomix) between 2013 and 2019 at a private Italian clinic (Genera Rome). The m-ER was investigated according to couples' previous reproductive history (previous live birth(s):no/yes; previous failed IVF cycle(s):-none/1/2/>2; previous miscarriage(s):none/1/>1). For PGT-A cycles following previous attempts (n=440/2676,16.4%), the m-ER was also investigated according to the former results (absence/presence of euploid blastocyst(s) in the previous cohort(s)).
Participants/materials, setting, methods: The m-ER followed a distribution different from a Gaussian (Kolgomorov-Smirnov and Shapiro-Wilk tests<0.01). Therefore non-parametric tests were adopted for the investigation (i.e. Mann-Whitney U or Kruskal-Wallis tests). Generalized-linear-models were adopted to adjust for confounders. Maternal age (mean:39.3±3.3yr) was the only variable significantly associated with the m-ER (partial-eta-squared:0.19,p<0.01 and power>0.99) and all the analyses were thus conducted among ranges of maternal age (<35yr,n=208 m-ER:66%±31%; 35-37yr,n=533 m-ER:58%±33%; 38-40yr,n=909 m-ER:43%±35%; 41-42yr,n=574 m-ER:28%±34%; >42yr,n=452 m-ER:17%±31%).
Main results and the role of chance: The m-ER was independent from previous live births (<35yr:no, n=195 67%±31%/yes, n=13 54%±36% p=0.2; 35-37yr:no, n=500 58%±32%/yes, n=33 58%±38% p=0.8; 38-40yr:no, n=795 43%±35%/yes, n=114 41%±33% p=0.6; 41-42yr:no, n=510 28%±34%/yes, n=64 29%±35% p=0.5; >42yr:no, n=387 17%±30%/yes=65 21%±36% p=0.6), as well as from the number of previous failed IVF cycles (<35yr:none, n=119 65%±31%/1, n=53 67%±30%/2, n=21 78%±23%/>2, n=15 54%±39% p=0.3; 35-37yr:none, n=301 59%±32%/1, n=102 59%±32%/2, n=62 57%±33%/>2, n=68 51%±35% p=0.4; 38-40yr:none, n=466 44%±35%/1, n=187 42%±34%/2, n=122 41%±34%/>2, n=134 40%±35% p=0.5; 41-42yr:none, n=277 27%±34%/1, n=138 27%±34%/2, n=76 35%±38%/>2, n=83 27%±29% p=0.4; >42yr:none, n=235 15%±28%/1, n=107 15%±28%/2, n=54 28%±39%/>2, n=56 23%±34% p=0.1) and/or miscarriages (<35yr:none, n=171 66%±31%/1, n=25 69%±27%/>1, n=12 63%±40% p=0.9; 35-37yr:none, n=416 58%±33%/1, n=75 56%±30%/>1, n=42 60%±36% p=0.6; 38-40yr:none, n=674 42%±35%/1, n=158 44%±34%/>1, n=77 44%±35% p=0.9; 41-42yr:none, n=404 29%±35%/1, n=99 29%±33%/>1, n=71 20%±27% p=0.3; >42yr:none n=324 18%±31%/1, n=79 17%±33%/>1, n=49 14%±25% p=0.8). Lastly, for the 440 cycles performed after former completed PGT-A cycles, the absence/presence of euploid blastocysts in the previous cohort(s) did not associate with the m-ER (<35yr:absence, n=5 69%±19%/presence, n=203 66%±31% p=0.3; 35-37yr:absence, n=23 40%±33%/presence n=510 59%±32% p=0.1 ; 38-40yr:absence, n=84 38%±33%/presence, n=825 43%±35% p=0.25; 41-42yr:absence, n=75 29%±35%/presence, n=499 28%±34% p=0.4; >42yr:absence, n=78 20%±31%/presence, n=374 17%±30% p=0.7).
Limitations, reasons for caution: Segmental and allegedly-mosaic aneuploidies were not reported. The sample size among the treatments following a former completed PGT-A cycle should be increased. The data should be confirmed from a multicenter perspective. For couples who experienced former IVF failures and/or miscarriages, the implantation rate after euploid blastocyst transfer should be investigated.
Wider implications of the findings: These data are critical to counsel infertile couples before and after PGT-A cycles. Apparently, their previous reproductive history does not affect the m-ER beyond the maternal-age-effect. For idiopathic repeated-IVF-failures and recurrent-pregnancy-losses, the causes might be related to endometrial receptivity issues. Yet, transferring only euploid blastocysts minimizes embryo-derived adverse reproductive outcomes.
Trial registration number: None

P-491 Cleavage biopsy, same results as trophectoderm biopsy but without mosaicism

C. Encinas[1], S. Novo[1], A. Brualla[2], Á. García-Faura[1], B. Marquès[1], F. García[1], C. Castelló[1], M. López-Teijón[1]

[1]Institut Marquès, Reproductive Medicine Service, Barcelona, Spain ; [2]Institut Marquès Ireland, Reproductive Medicine Service, Clane, Ireland

Study question: Analysing the evolution of PGT-A, does the biopsy at blastocyst stage bring great advantages?

Summary answer: Both cleavage and blastocyst stage biopsy provide optimal and equivalent clinical results.
What is known already: Recently, trophectoderm biopsy has been considered the gold standard for Preimplantational Genetic Testing (PGT). The main reason is that the higher number of analysed cells, the better representation of embryo's chromosomal constitution should be achieved. However, this technique has also made the mosaic embryo diagnostic group appear, a result that can be considered as ambiguous. The possibility of getting a confusing result is being used by some groups as an argument to challenge the usefulness of blastocyst biopsy and PGT-A. This study aims to assess the differences in clinical outcomes between treatments were cleavage or trophectoderm biopsies have been performed.
Study design, size, duration: This is a retrospective study containing data from 681 single blastocysts transfer PGT-A cycles between 2017 and 2019 using Next Generation Sequencing (NGS). On one hand, D3-group: 229 cycles with cleavage stage embryos biopsied at day 3 of culture, cultured up to blastocyst stage and fresh transferred. On the other hand, D5/6-group: 452 cycles, with embryos cultured to blastocyst stage, biopsied and vitrified.
Participants/materials, setting, methods: In both groups, embryos were cultured using single step media in time-lapse incubators up to blastocyst stage. Patient characteristics and demographic data were homogenous and similar between both groups. The number of embryos biopsied, blastocyst rate, results of *NGS* analysis and pregnancy and miscarriage rates were compared between both groups. The Chi-square test was used for statistics.
Main results and the role of chance: D3-group had significantly more biopsied embryos (n=1097/1287; 85.2%) than D5/6-group (n=1715/3233; 53%; p<0.05). However, the good quality blastocyst rate was equivalent between groups (D3=50.2%; D5/6=53%; p=0.086), demonstrating that cleavage stage embryo biopsy did not compromise embryonic development.

To compare NGS results between groups, all biopsied embryos were included in D5/6 group, while in D3-group only the biopsied embryos with good quality in day 5/6 were considered (≥3BB Gardner score). Aneuploidy rates proved to be similar (D3:48%; D5/6:46.8%; p=0.262) as well as the non diagnostic result (D3=5.1%; D5/6=1.2%; p=0.091). Nevertheless, there were statistically significant differences between euploidy rates, being higher in D3-group (46.9%) than in grouP-D5/6 (34.5%; p<0.05). After genetic counselling, and considering some mosaic embryos as transferable, a total of 45.3% of embryos could be considered for transfer matching the values obtained in D3-group.

In both groups, a single blastocyst was transferred. There were 144 embryo transfers in D3-group and 262 in D5/6 group. Pregnancy rates (D3=68.8%; D5/6=72.5%; p=0.152), ongoing pregnancy rates (D3:=52.8%; D5/6=52.3%; p=0.411), and miscarriage rates (D3=8.1%; D5/6=8.4%; p=0.356), were equivalent between groups.
Limitations, reasons for caution: Although the considerable data set provided, live birth rates should be analysed. Every D3-group embryo was transferred in the fresh cycle, but all D5/6-group embryos were transferred in a cycle were no ovarian stimulation was performed. This could be a source of bias.
Wider implications of the findings: Considering that pregnancy and miscarriage rates were similar, both strategies can coexist and it is not mandatory to biopsy at blastocyst stage. As it is done in our clinic, each case should be evaluated to decide which is the best strategy for the patient.
Trial registration number: doesn't apply

P-492 Mitoscore in a euploid embryo- can it be a game changer ?

S.S. Garimella[1], K. Mantravadi[1], D.R. Gedela[1]

[1]oasis fertility, reproductive medicine, hyderabad, India

Study question: Does mitoscore value predict implantation potential of an embryo and have an impact on antenatal outcomes and neonatal birth weight?
Summary answer: Mitoscore has no role in predicting implantation potential of an embryo, but high mitoscore was associated with hypertensive disorders in pregnancy and lower birth weight.
What is known already: Mitoscore is a mitochondrial biomarker which is an indicator of energy status of embryo. Some studies indicate that increase in the mitochondrial DNA (mtDNA) in the embryo is indicative of insufficient level of energy and a low implantation potential. But recently there is a debate about the usefulness of mitoscore as a predictor of successful implantation .Therefore, more research is needed to further our understanding of the clinical usefulness of mitoscore as a biomarker for embryo viability
Study design, size, duration: This is a retrospective study involving analysis of 132 term babies born from transfer of a single euploid blastocyst from February 2018 to march 2019 at our center
Participants/materials, setting, methods: We performed preimplantation genetic testing for aneuploidy (PGT A) for patients with advanced maternal age, recurrent pregnancy losses and recurrent implantation failure.Trophectoderm biopsy was done and the samples were analysed by comprehensive chromosomal screening. Based on mitoscore value two groups were formed, group A (< 25) group B (> 25). Clinical pregnancy rates(CPR), live birth rates (LBR), hypertensive disorders in the antenatal period, birth weights and anomaly rates in the two groups were compared.
Main results and the role of chance: There was no difference in baseline characters between the two groups. Out of 132 euploid blastocysts 64 (48.4%) of the blasts had a mitoscore< 25 and 68 (51.6%) had a mitoscore of > 25. There was no statistically significant difference in CPR (68.7 % vs 66.1%) and LBR (57.8% vs 55.8%).Hypertensive disorders in the antenatal period were significantly higher in the group with mitoscore > 25 (37.6% in group B vs 12.2% in group A). %). Birth weights of babies in the group with mitoscore more than 25 (2.54 +/- 0.46) was significantly lower when compared to the group with mitoscore less than 25 (2.19 +/- 0.36) (p=0.025). There was no difference in anomaly rates in both the groups
Limitations, reasons for caution: This is a retrospective data and sample size was small and indications of PGT A are still debatable. Further research is necessary.
Wider implications of the findings: This study provides evidence that mitoscore has no role in predicting the implantation potential of an embryo. Higher mitoscore embryos were associated with hypertensive disorders in pregnancy and lower birth weights. Therefore further research is required to understand the implications of mitoscore and its effect on reproductive outcomes
Trial registration number: not applicable

P-493 Prospective analysis of multiplatform preimplantation genetic testing for aneuploidy (PGT-A) confirmation of trophectoderm re-biopsy from aneuploid embryos and corresponding whole embryos

M. Abae[1], N. Arrach[2], Y. Ying[3], X. Zhang[4], A. Goodman[5], K. Abae[6]

[1]Fertility & Genetics, Reproductive Endocrinology, Plantation- FL, U.S.A. ;
[2]Progenesis, Progenesis, San Diego, U.S.A. ;
[3]Univeristy of Soujht Florida, Obstetrics & Gynecology, Tampa, U.S.A. ;
[4]Fertility & Genetics, Embryology, Plantation, U.S.A. ;
[5]NXGENMDX, Pgt, Grand Rapids, U.S.A. ;
[6]Fertility & Genetics, Fertiility & Genetics, Plantation, U.S.A.

Study question: How do results of PGT-A compare between different contemporary testing platforms, next generation sequencing (NGS) and single nucleotide polymorphism (SNP) from trophectoderm (TE) re-biopsy samples and corresponding whole embryos (EM)
Summary answer: SNP had moderate correlation with NGS. Discrepancy between TE and whole EM results was not associated with platforms, but to sample variability and potentially mosaicism.
What is known already: PGT-A improves successful pregnancy outcomes following elective single embryo transfer. However, significant clinical uncertainty remains about the accuracy of mosaicism in embryos undergoing PGT-A testing. Furthermore, re-biopsy of abnormal embryos have occasionally led to euploid results adding new challenges to the PGT-A management and patient expectation.
Study design, size, duration: Thirty pre-tested aneuploid embryos that were donated by couples who had a previously confirmed viable pregnancy after transfer of single euploid embryo following PGT-A. Discarded genetically abnormal embryos that were consented for donation for research were analyzed in this exempt IRB approved blinded study. The inclusion criteria did not consider factors such as cause of infertility, age, race, or ethnicity. This study excluded any untested or genetically normal embryos.
Participants/materials, setting, methods: Thirty pre-tested aneuploidy embryos with SNP that were donated for this study were re-biopsied. TE and the corresponding EM were loaded separately. Sixty blinded samples were

subject to whole genome amplification followed by NGS. Each DNA sample was tested with two sequencing platforms: VeriSeq-PGS and Reproseq.

Main results and the role of chance: Of 30 embryos, 23 were successfully amplified/sequenced in both TE and EM. The two NGS platforms had 100% correlation when same source DNA is used. Each sequencing platform showed 96% correlation between TE and EM. SNP and NGS had 79% correlation at TE level and 84% at EM. Discrepancy between TE and whole EM results was not associated with the platforms, but to sample variability, potentially due to mosaicism. There was 17.4% (4 out of 23) of re-biopsied TE and whole EM found to be euploid by the two NGS platforms compared to SNP. This discrepancy could be related to the technology used, biopsy quality or to embryonic mosaicism.

Limitations, reasons for caution: The two NGS platforms were performed on the same DNA, while SNP array was performed with separate biopsy. Difference in biopsy quality and/or potential mosaicism can lead to a discrepancy between NGS and SNP.

Wider implications of the findings: PGT-A has shown to improve pregnancy outcomes with a single embryo transfer. However, the choice of PGT-A technology platform presents a challenge from genetic counselling perspective. The impact of potential mosaicism, PGT-A technological limitation, and variability across biopsies should be taken into account when counseling patients with PGT-A.

Trial registration number: Not Applicable

P-494 Clinical exome sequencing from peripheral blood reveals novel gene variants to prevent ovarian failure

I. Henarejos Castillo[1,2], A. Aleman[1], B. Martinez-Montoro[3], F. Javier Gracia[4], P. Sebastian-Leon[1,5], J.A. Remohl[2,6], A. Patiño[4,7], P. Royo[3], G. Alkorta-Aranburu[4], P. Diaz-Gimeno[1,5]

[1]IVI-RMA IVI Foundation, Department of Genomic & Systems Reproductive Medicine, Valencia, Spain ;
[2]Universidad de Valencia, Department of Pediatrics- Obstetrics- and Gynaecology, Valencia, Spain ;
[3]IVI-RMA Pamplona, Reproductive Medicine, Pamplona, Spain ;
[4]CIMA Lab diagnostics, CIMA lab, Pamplona, Spain ;
[5]Instituto de Investigación Sanitaria Hospital Universitario y Politécnico La Fe, Instituto de Investigación Sanitaria Hospital Universitario y Politécnico La Fe, Valencia, Spain ;
[6]IVI-RMA Valencia, Reproductive Medicine, Valencia, Spain ;
[7]Universidad de Navarra, Laboratorio de Pediatría- Unidad de Genética Clínica, Pamplona, Spain

Study question: Could the identification of specific sequence variants in DNA stratify individuals with ovarian failure?

Summary answer: This prospective study has discovered 66 DNA variants associated with ovarian failure.

What is known already: Around 1% of women under the age of 40 develop premature ovarian insufficiency (POI), which includes accelerated attrition of the ovarian follicle reserve and amenorrhea. An unknown percentage of women suffer occult ovarian failure, a less severe form of ovarian insufficiency that increases infertility due to a diminished ovarian reserve. In most cases, the genetic cause or origin of this heterogeneous and complex disorder is unknown. Interestingly, whole exome sequencing based studies have started identifying new POI-associated sequence variants even though most of those studies suffer from lack of control or small sample size.

Study design, size, duration: A 150 case-control study was conducted between 2017 and 2019: 118 cases diagnosed with primary ovarian insufficiency (n=35) or occult ovarian failure (n=83) and 32 controls based on AMH levels and/or antral follicle counts. Next, whole-exome sequencing of DNA from 150 peripheral blood samples was performed using SureSelect Clinical Research Exome V2 (Agilent Technologies) and Illumina sequencing. The study was approved by the institutional review board of Instituto Valenciano de Infertilidad (1709-PAM-090-PR).

Participants/materials, setting, methods: In order to identify variants more likely associated with ovarian failure, we excluded variants with a population frequency higher than 5% - considering the negative impact of variants associated with ovarian failure during natural selection. In addition, only variants in genomic positions with >100x were considered. Furthermore, variants were filtered according to their absence/presence in IGSR, to identify new variants and only non-synonymous variants with consequence at protein level were considered for case-control comparison.

Main results and the role of chance: Genes previously associated with ovarian failure accumulated less gene variants than any selection from the total of the exome (t test, p value =2.2e-16). Analysis of the clinical exome (18311 genes) revealed more than 47,000 variants were patient specific, therefore associated with individual genetic variation. We highlighted 66 novel variants that were at least shared by 10 % of cases and absent in controls, as a criterion to be related to ovarian failure. This profile of 66 variants altogether identified the 100% of cases stratifying into two main types of ovarian failure, finding inside one of them two clear subtypes. With two variants, DNAH6, a gene related to microtubule activity, was the major contributor to the stratification. Furthermore, 6 of these 66 variants were presented in at least 20 cases, one particularly affecting 31 cases of ovarian failure including both primary ovarian insufficiency and occult ovarian failure. Genes and biological processes affected by these new variants included: MUC6 for cytoprotection of epithelial surfaces; BRPF3, involved in chromatin organization; AP5M1,which could play a role in apoptosis; CRISPLD2, that promotes extracellular matrix assembly; GALNS, participates in degradation of glycosaminoglycans; and MCM5, a component of the complex needed for DNA replication initiation.

Limitations, reasons for caution: To prove the preventive value of this variant profile, an independent clinical validation should be performed. A higher sample size would help in the patient stratification of subtypes of ovarian failure due to the genetic heterogeneity of this disorder; however, we have detected a common variant in 31 cases.

Wider implications of the findings: This study elucidates the underlying genetic cause of idiopathic ovarian failure, identifying new variants by blood sequencing that could become a preventive biomarker and create a new taxonomy for this disorder. Additionally, our results highlighted that studies based only on disease associated genes could overshadow discovery of new variants.

Trial registration number: Research supported by IVI Foundation, IVI-RMA Global. I. Henarejos-Castillo is financially supported by the Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital (ACIF/19/148). Research co-financed by Navarra Goverment and European Regional Development's funds (FEDER): 0011-1365-2017-000265. Begoña Montoro is financially supported by Navarra Goverment: 0011-1408-2018-000011

P-495 Correlation between blastocyst morphology, euploidy and implantation in a PGT-A program of 1312 screened blastocysts. Is it worth biopsing a grade C embryo?

C. Escriba[1], M. Alaves[1], M. Benavent[1], C. Miret[1], A. Garcia[1], N. Costa-Borges[2], G. Calderon[2], J. Crespo[1], J. Teruel[1]

[1]Juana Crespo, IVF Laboratory, Valencia, Spain ;
[2]Embryotools, Research and quality, Barcelona, Spain

Study question: What is the reproductive potential of grade C blastocysts? Are we really helping the patients by biopsing them?

Summary answer: Grade C blastocysts have significantly poorer reproductive potential than grade A and B blastocysts regarding aneuploidy and implantation rates but still result in ongoing pregnancies.

What is known already: Several studies show the benefit of trophectoderm biopsy combined with chromosomal screening, a good vitrification program and subsequent transfer of euploid embryos. However, the indications as to when to biopsy poor quality embryos and their clinical contribution to patients is still unclear or controversial.

Some studies suggest that grade C embryos have a higher aneuploidy rate, but euploid ones have a similar implantation rate to better quality embryos. However, this contradicts the establish fact that embryo quality is predictive of implantation.

Study design, size, duration: This is a retrospective observational study performed in a private centre between May 2017 and September 2019. The study includes the data analysis of 1312 blastocysts undergoing PGT-A (mean maternal age, 39.4 ± 3.76 years) obtained from 270 patients following 339 PGS cycles. 232 euploid blastocysts were transferred in 178 frozen embryo transfers. Of this, it was possible to track the implantation outcome of 226 of them.

Participants/materials, setting, methods: PGT-A was offered to patients of advanced maternal age and/or with repeated IVF failures. Patients underwent

ICSI cycles with day 3 assisted hatching. Trophectoderm biopsies were performed on day 5 and/or day 6 embryos, with laser assistance.
Blastocyst morphology was scored in 3 groups: A: excellent (AA, AB, BA), B: good (BB), C: average and poor-quality embryos (BC, CB, CC). (Gardner-Schoolcraft classification)
Relationships between these groups regarding euploidy, implantation and miscarriage rates were assessed.
Main results and the role of chance: The euploidy rate was 38.2 (n=157), 29.7(n=853) and 26.8% (n=302) in the A, B and C blastocyst morphology groups, respectively, showing that in group A there were significantly more euploid embryos than in groups B and C. There are no differences between the mean age of the 3 groups. (38.16, 38.26 and 38.46 years, respectively).

The mosaicism rate does not differ between groups. (14, 15 and 13.6% in A, B and C groups, respectively).

Chi-square tests were used to assess the relationship between blastocyst morphology and euploidy rates and between blastocyst morphology and implantation/miscarriage rates of euploid blastocysts.

The implantation rate of euploid blastocysts was 80 (n=45),61.15 (n=139) and 35% (n=42) in the A, B and C blastocyst morphology groups, respectively, showing high significant differences among the three groups.

Focusing only on poor-quality (CC) blastocysts within the grade C embryos population we observe that the implantation rate drops to 25% (n=24) from the 35% of overall implantation rate of the group (BC, CB, CC), highlighting the importance of embryo quality as a predictor of the implantation potential even if euploid.

Miscarriage rates were inversely correlated to quality (8 (n=36), 13 (n=85) and 26% (n=15)) but did not achieve statistical significance.
Limitations, reasons for caution: The study is limited by its retrospective nature and the low number of grade C blastocysts transferred as they are the last option for transfer. Additionally, it is common to transfer more than one grade C embryo to increase the chances of pregnancy, losing implantation track of some of them.
Wider implications of the findings: It is uncontested that grade C embryos have a lower reproductive potential, but we managed to quantify this potential, providing clinicians with useful information to manage the cycle and inform patients accordingly.
Trial registration number: Not applicable

P-496 The Structural Rearrangement-specific Characteristics Have Limited Effect on Ploidy Status in Young Couples Undergoing Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR) by aCGH/NGS

M. Gultomruk[1], F.K. Boynukalin[2], C. Rubio[3], L. Rodrigo[3], E. Turgut[2], Z. Yarkiner[4], S. Ecemis[5], G. Karlikaya[2], N. Findikli[1], M. Bahceci[2]

[1]Bahceci Fulya IVF Centre, IVF Laboratory, Istanbul, Turkey ;
[2]Bahceci Fulya IVF Centre, Infertility Clinic, Istanbul, Turkey ;
[3]Ignomix S.L, Reproductive Genetics, Valencia, Spain ;
[4]Cyprus Science University, Biostatistics, Kyrenia, Cyprus ;
[5]Bahceci Ankara IVF Centre, Infertility Clinic, Ankara, Turkey

Study question: What are the possible effects of carrier and rearrangement-specific characteristics on the ploidy status of embryos and presence of interchromosomal effect(ICE) in cases undergoing PGT-SR?
Summary answer: Euploidy and ICE rates are similar in Robertsonian and reciprocal translocations. Euploidy rates are independent from carrier and structural rearrangement-specific characteristics, except female age.
What is known already: Structural chromosome rearrangements such as reciprocal translocations (RecT), Robertsonian translocations (RobT) and inversions are associated with increased risk of fertility problems, recurrent miscarriages and progeny congenital abnormalities due to the production of unbalanced gametes during meiosis. It has long been suggested that chromosomes with structural rearrangements can also impair the segregation of other chromosomes which is known as ICE. ICE has previously been evaluated in several studies, but the results are conflicting. The limited number of chromosomes analyzed in fluorescence in situ hybridization(FISH) technique and blastomere biopsy could be the main drawbacks of most of the published studies evaluating ICE.
Study design, size, duration: This retrospective study includes 95 RecT (124 cycles) and 36 RobT (49 cycles) carriers undergoing PGT-SR between March 2016-July 2019. Array Comparative Genomic Hybridization (aCGH) or Next Generation Sequencing (NGS) were the techniques used coupled with trophectoderm biopsy (TB). Only cases with female age under 37 were included. A total of 532 blastocyst embryos (female RecT;200, male RecT;176, female RobT;78 and male RobT;78) were evaluated.
Participants/materials, setting, methods: One hundred and seventy-three PGT-SR cycles with 532 blastocyst-stage embryos were evaluated according to the gender of the carrier partner, the type of rearrangement, chromosomes involved in the translocations as well as the position of breakpoints on the incidence of balanced/unbalanced embryos and ICE. Multi-level generalized linear mixed model was used to for statistical comparisons of the probability of achieving a euploid embryo.
Main results and the role of chance: The median female and male ages, retrieved oocytes, matured oocytes, number of fertilized oocytes and number of biopsied embryos were similar among the female RecT and RobT, and male RecT and RobT carriers. The euploidy rate was also similar in RobT compared with RecT carriers [57/156(36.5%) vs 112/376(29.8%), p=0.127]. Pure ICE (aneuploid balanced) rate was significantly higher in RobT [48/156(30.8%) vs 53/376(14.1%), p<0.001]. In contrast, combined ICE (aneuploid unbalanced) was significantly higher in RecT [72/376(19.1%) vs 16/156(10.3%), p=0.011]. No significant differences were observed in the total percentage of pure ICE plus combined ICE between RobT and RecT [64/156(41%) vs 125/376(33.2%), p=0.088]. A gender-based subgroup analysis also shown no differences in euploidy rate between male RecT and female RecT carriers [58/176(33%) vs 54/200(27%), p=0.207] and male RobT and female RobT carriers [28/78(35.9%) vs 29/78(37.2%), p=0.86]. No significant differences were observed in euploidy rates, pure ICE and combined ICE according to the length of the translocated fragment, the chromosome group, and the position of the breakpoints in RecT cases. Multi-level generalized linear mixed model using binomial distribution revealed that and female age was the factor related to the incidence of euploid embryo.
Limitations, reasons for caution: The size number, the retrospective nature of the study and the absence of age-matched controls are the main limitations.
Wider implications of the findings: Limited studies evaluated PGT-SR based on TB and 24 chromosomes analysis. Reported studies included limited embryo yield; female age, which caused bias, is not restricted. Our data provide a deeper insight about the effect of the translocation type, carrier-gender, chromosomes involved in rearrangement and position of breakpoints on PGT-SR results.
Trial registration number: None

P-497 Genetic and Clinical Outcomes of Preimplantation Genetic Testing for Aneuploidy (PGT-A) by Using Next Generation Sequencing (NGS) in Young Couples With Idiopathic Recurrent Miscarriage

F.K. Boynukalin[1], M. Gultomruk[2], E. Turgut[3], S. Ecemis[4], Z. Yarkiner[5], R. Abali[3], G. Karlikaya[3], N. Findikli[6], M. Bahceci[7]

[1]Bahceci Health Group, Reproductive Endocrinology, Istanbul, Turkey ;
[2]Bahceci Fulya IVF Centre, IVF Laboratory, Istanbul, Turkey ;
[3]Bahceci Fulya IVF center, Infertility Clinic, Istanbul, Turkey ;
[4]Bahceci Ankara IVF Center, Infertility Clinic, Ankara, Turkey ;
[5]Cyprus Science University, Biostatistics, Kyrenia, Cyprus ;
[6]Bahceci Fulya IVF Center, IVF Laboratory, Istanbul, Turkey ;
[7]Bahceci Fulya IVF Center, Infertility, Istanbul, Turkey

Study question: Are clinical outcomes of preimplantation genetic test for aneuploidy(PGT-A) satisfactory in idiopathic recurrent miscarriage(iRM) and is there a characteristic distribution for aneuploidy screening data?
Summary answer: Although ~50% live-birth per embryo transfer(ET) is acquired, high miscarriage rate(MR) is observed in iRM with PGT-A. Aneuploidy type differs compared to product of conception.

What is known already: Aneuploidy account for 42% of miscarriages in RM. The theoretical goal of PGT-A was to identify and transfer euploid embryos and decrease the rate of miscarriage. There is paucity of data regarding the role of PGT-A in RM. Although, several retrospective analysis performed on RM patients demonstrated a trend toward decreased MR and increased live birth rate(LBR), such results are usually blurred and biased by the presence of an additive aneuploidy risk as advanced maternal age. In addition, these studies used chromosomes analyzed in fluorescence in situ hybridization(FISH) technique and blastomer biopsy which are the main drawback of the studies.

Study design, size, duration: This is a retrospective single center study to analyze the PGT-A results, using trophectoderm biopsy and NGS, of iRM cases in which female age is <35. Data of 133 patients undergoing PGT-A with their 423 blastocyst embryos between February 2016-January 2019 were evaluated. Patients were sub-grouped according to the number of previous miscarriages as; 2, 3, and >3. The effect of patient and embryo characteristics on ploidy status, LBR and MR were assesed.

Participants/materials, setting, methods: Multi-level generalized linear mixed model was used to analyze the effect of previous live birth, previous number of miscarriages, body mass index, female age, male age and cycle characteristics on probability of achieving a euploid embryo. Binary Logistic Regression was used to analyze affect of factors on abortion compared to live birth. The distribution of monosomies, trisomies and partial aneuploidies affecting each chromosome were detected in embryo yield.

Main results and the role of chance: The overall incidence of euploid embryo was found to be 62.4%(264/423). The distribution of aneuploidy type was as; monosomy 27%(43/159), trisomy 22%(35/159), segmental 17%(27/159), double aneuploidy 15.7%(25/159), complex aneuploidy 11.9%(19/159) and chaotic 6.3%(10/159). The most frequently involved chromosomes in aneuploidies were 16(14%), 21(10.1%) and 22(9.4%). The euploidy rate of embryos was not statistically different between 2, 3 and >3 number of miscarriages (64.7%(124/207), 59.4%(82/138), 61.5%(48/78); respectively, p=0.598). Multi-level generalized linear mixed model revealed that female age was the only significant factor that effects the probability of euploidy (OR:0.87, 95%CI:0.78-0.98, p=0.009). Clinical pregnancy rate (CPR), LBR and MR for patients per ET was 64.3%(83/129), 48.8%(63/129) and 24.1%(20/83) respectively. Although there is a trend toward increased MR with increasing number of previous miscarriage, CPR, LBR and MR were not significantly different in subgroups regarding 2, 3, >3 (CPR: 62.3%(43/69), 75.7%(28/37), 52.2%(12/23), p=0.16; LBR: 52.2%(36/69), 51.4%(19/37), 34.8%(8/23), p=0.33; MR: 16.3%(7/43), 32.1%(9/28), 33.3%(4/12), p=0.23 respectively). MR is higher than reported studies for infertile patients in the PGT-A and lower than expected MR for RM patients with natural conception. Binary logistic regression analysis showed that BMI (p=0.004, OR:1.22, 95%CI[1.06-1.4]), embryo quality (poor quality, p=0.082, OR:4.6, 95%CI[1.2-17.1]) were significantly correlated with miscarriage.

Limitations, reasons for caution: The retrospective nature of the study is the main limitation. In addition, the study did not contain a control group.

Wider implications of the findings: Until now, there is paucity of data to recommend PGT-A as part of routine use for RM. However the data are compelling that this technology can enhance outcome by reducing the risk of clinical miscarriages and ongoing aneuploidy pregnancies. Well-designed studies is needed to analyze efficacy and safety of treatment.

Trial registration number: not applicable

P-498 Relationship between both euploidy and thawing survival rates and embryo quality, day of biopsy or hatching status in Preimplantation Genetic Testing cycles

M. Florensa Bargalló[1], A. Cladellas Artola[1], M. Bellés Fernandez[1], M. Jimenez Blasco[1], J. Herreros Cuesta[1], H. Huete Ferriz[1], N. Garrido Puchalt[2], M. Esbert Algam[1]

[1]IVI Barcelona, IVF laboratory, Barcelona, Spain ;
[2]IVI Foundation, Research administration, Valencia, Spain

Study question: In blastocyst biopsy cycles, is there any association between embryo quality, biopsy day and embryo hatching status and both embryo ploidy or post-thawing survival rates?

Summary answer: Good embryo quality is related to euploidy while day-6 biopsied embryos and hatched blastocysts have fewer chances to survive after freezing.

What is known already: In Preimplantation Genetic Testing (PGT) cycles, blastocyst formation has long been related to euploidy and some authors have detected differences in the ploidy depending on the embryo quality or whether the biopsy has been performed on day-5, day-6, or day-7 of development. However, very little has been published about differences in the survival rate related to the quality of the Inner Cell Mass (ICM) and the trophectoderm (TE), the day of biopsy or the hatching status of the embryo.

Study design, size, duration: Retrospective study including 2538 biopsied blastocysts from 856 PGT cycles performed in the same center between February 2018 and October 2019. Biopsied embryos were divided according to ICM and TE quality Gardner's evaluation (Best AA; Good AB, BA, BB; Poor AC, CA, BC, CB, CC) and the day of biopsy (day-5 or day-6). Completely hatched blastocysts (HB) were analyzed separately. The independent relationship between each group and both euploidy and survival rate was assessed.

Participants/materials, setting, methods: For each cycle, serial biopsies were performed depending on the herniation status of each embryo on day-5 or on day-6 of development. TE biopsy was immediately followed by vitrification of all biopsied embryos. Comprehensive chromosome screening PGT was performed using high resolution next generation sequencing (NGS) methodology. Embryos were thawed and replaced in case of both normalcy and survival evidence following an endometrial preparation cycle. Statistical report was performed under R version 3.6.1. (2019-07-05).

Main results and the role of chance: A total of 2534 embryos resulted informative after genetic analysis (99.84%). Embryo ploidy showed a relationship with embryo quality as significant differences were found among the percentage of euploid embryos of the three quality groups (Best 61.56% n=294; Good 44.52% n= 1642; Poor 31.09% n= 595) (p<0.001). Euploidy did not correlate with the biopsy day (hence, the day of herniation) independently of the embryo quality or with the hatching status of the embryo. Thus, no differences were found between the euploidy rate of day-5 (44.15%; n=1838) and day-6 (40.72%; n=679) (odds ratio [OR]=0.87; p=0.1191) or between the chromosomal status of the hatched (47.19%; n=310) versus non-hatched embryos (42.79%; n=2228) (OR 0.84; p=0.1375). Regarding survival rate, results showed no relationship with embryo quality even if a trend was detected against Poor embryo quality group compared to Best (OR=0.38; p=0.1670) and Good (OR=0.36; p=0.0779). On the other hand, non-hatched day-6 biopsied embryos had lower survival rates (82.19%) than non-hatched day-5 embryos (91.46%) (OR=0.12; p=0.0111) independently of the embryo quality. Concerning the hatching status of the embryo, HB had fewer chances to survive (69.01%) than non-hatched blastocysts (91.27%) (OR=4.69; p<0.001).

Limitations, reasons for caution: This study is limited by its retrospective nature. Even if embryologists do pass routine evaluation controls, qualitative blastocyst morphology grading is always confined by its subjectivity.

Wider implications of the findings: Knowing that embryo quality correlates to euploidy can help improving the counselling to PGT and non-PGT patients. Furthermore, finding that day-6 non-hatched blastocysts and HB have fewer chances to survive can lead to a more continuous evaluation of the embryos on day-5 in case of blastocyst biopsy.

Trial registration number: not applicable

P-499 Polar body analysis in preimplantation genetic testing for monogenic diseases (PGT-M) – a genome wide approach for haplotype phasing and assessment of ploidy

P. Forsblom[1], J. Pricelius[1], M. Stecher[1], C. Bayerl[1], D. Gutknecht[2], B. Poost[3], Y. Charron[4], C. Meyer[5], A. Jantke[4], D. Noss[3], M. Bals-Pratsch[2], C. Nevinny-Stickel-Hinzpeter[1]

[1]SYNLAB Human Genetics Munich, Preimplantation diagnostics and chromosomal microarrays, Munich, Germany ;
[2]Profertilita Centre for Reproductive Medicine, IVF clinic, Regensburg, Germany ;
[3]Centre for Reproductive Medicine, IVF clinic, Munich, Germany ;

[4]Centre for Assisted Fertility Treatment and Fertility Preservation, IVF clinic, Berlin, Germany ;
[5]Cryos International, Denmark ApS, Aarhus, Denmark

Study question: Can single nucleotide polymorphism (SNP) arrays be successfully utilized on polar bodies for simultaneous PGT-M and assessment of the chromosomal status of the oocyte?
Summary answer: Genome-wide SNP array provide a nearly universal tool for PGT-M on polar bodies. The oocyte ploidy can be assessed in the same analytical procedure.
What is known already: Polar bodies can be sourced as an alternative sample material for PGT-M when the analysis of blastomeres or trophectoderm cells is not possible due to ethical, medical, religious or legal reasons. Traditionally, the analysis has been based on short tandem repeats (STRs) and linkage analysis which is time consuming and costly as the assay has to be developed for each family separately. As only the disease locus is assessed in the STR-based linkage analysis the chromosomal status of the oocyte remains unclear. Thus, there is room for improvement in the current methodology.
Study design, size, duration: Retrospective study in a setting of a commercial diagnostic laboratory. The phenotypes segregating in the families were inherited as autosomal dominant (inherited breast and ovarian cancer, spinocerebellar ataxia type 3), autosomal recessive (puryvatkinase deficiency) and X chromosomal (ornithine transcarbamylase deficiency, Renpenning syndrome) traits. Altogether five patients were included in the study. The work was conducted between February 2018 and December 2019.
Participants/materials, setting, methods: Sequentially biopsied polar bodies from oocytes fertilized with ICSI were subjected to whole genome amplification (Repli-g Single Cell Kit; Qiagen). Genome-wide genotyping of the amplified samples as well as DNA samples of the mother and at least one reference individual was carried out (Human KaryomaP-12 SNP array; Vitrolife). A direct analysis of the disease-causing variant was included in the assay when applicable. BlueFuseMulti software (Illumina) with modified settings was utilized for the data analysis.
Main results and the role of chance: Here we report the successful use of genome-wide genotyping on polar bodies allowing the simultaneous assessment of the maternally inherited disease and the oocyte ploidy. Polar body sets of 19 zygotes were analysed: eleven zygotes were non-affected, seven affected and one couldn't be interpreted because the second polar body delivered no data. The genome-wide analysis showed aneuploidy in four of the non-affected zygotes. Three patients had one zygote suitable for culture and transfer whereas two patients had two suitable zygotes. One double-embryo transfer and two single embryo transfers ensued resulting in one clinical pregnancy and birth of a healthy baby. Two non-affected zygotes didn't survive warming. One patient with one suitable zygote is awaiting for warming and transfer.

The set-backs in polar body analysis are considerable. When the disease is inherited in autosomal recessive or X-chromosomal manner the sole use of polar body analysis results in wasting of a significant number of zygotes as paternally inherited alleles and the sex of the embryo cannot be determined. In comparison to trophectoderm cells the analysis of polar bodies inherently contains a higher fall out rate, although in this patient series only two polar bodies failed the amplification or analysis.
Limitations, reasons for caution: This is a retrospective study with a small sample size. Thus, further studies are needed to establish the clinical and diagnostic utility of the procedure. Only maternally inherited diseases can be analysed and both polar bodies are needed for reliable diagnostics.
Wider implications of the findings: In situations where PGT-M on embryonal cells isn't allowed or desired polar body analysis is a worthy alternative. In these cases SNP arrays allow the testing of a wide range of genetic diseases with the simultaneous detection of oocyte ploidy, particularly of interest for patients of advanced maternal age.
Trial registration number: Not applicable

P-500 Novel compound heterozygous mutations in WEE2 gene is associated with fertilization failure: a case report about an infertile woman

Y. Tian[1], B. Xiaohong[2]

[1]Tianjin Medical University General Hospital, Department of Gynecology and Obstetrics-, Tianjin, China ;
[2]Tianjin Medical University General Hospital, Department of Gynecology and Obstetrics, Tianjin, China

Study question: To identify the genetic cause of repeated fertilization failure even after ICSI combined with artificial oocyte activation (ICSI-AOA) in a non-consanguineous family.
Summary answer: With whole-exome sequencing, a novel compound heterozygous mutation in *WEE2* gene was identified in an infertile female who experienced with fertilization failure after ICSI-AOA
What is known already: The genetic factors play an important role in oocyte activation defect and likely result in repeated human fertilization failure even after ICSI-AOA. In mouse oocyte at metaphase II stage, Wee2 inhibited phosphorylation of Cdc2, which was required for metaphase II exit. Down regulation of Wee2 during egg activation leads to failure of pronucleus formation. Recently, mutations in *WEE2* were identified to result in failure of pronuclei formation and human fertilization in infertile women.
Study design, size, duration: We have characterized a patient with 2-year history of primary unexplained infertility belonging to a non-consanguineous family from China. During 3 assisted reproduction attempts (IVF+ rescue ICSI, ICSI and ICSI-AOA), the woman presented a repeated fertilization failure for MII oocyte retrieval after controlled ovarian hyperstimulation. We performed whole-exome sequencing and sanger sequencing in the patient and her family members.
Participants/materials, setting, methods: Genomic DNA samples of the patient and her family's members were extracted from peripheral blood. Whole-exome capture and sequencing were performed following the standard protocols provided by BGI Genomics (BGI-Shenzhen). The sequencing depth of WES was 100× . The pathogenicity of variants was assessed by four software: SIFT, PolyPhen2, Mutation Taster and Human Splicing Finder. The structure prediction of wide type and mutant type WEE2 were performed by PyMol software.
Main results and the role of chance: Three ART cycles were conducted for this couple and even ICSI-AOA did not rescue fertilization failure phenotype. Aneuploidy or pathogenic microdeletion/microduplication (>100Kb) were not found in the couple. We identified a novel compound heterozygous mutation c.1535+3A>G and c.946C>T (p. Leu316Phe) in the *WEE2* gene in female proband who experienced fertilization failure. The allele frequencies of variation c.1535+3A>G and c.946C>T were not found in the 1000genome database or ExAC database. Trios analysis revealed that the detected variations follow an autosomal recessive pattern. Variation c.1535+3A>G in *WEE2* was predicted to break WT donor site and probably affects splicing. With SIFT, PolyPhen-2 and Mutation Taster software, it is predicted that the missense mutation c.946C>T (p. Leu316Phe) of *WEE2* is pathogenic. Prediction with PyMOL software indicated that the mutation could change the structure of WEE2 protein.
Limitations, reasons for caution: The functional analysis of oocytes is not performed yet to detect the local expression of this variant. A causal relationship between the mutation and the fertilization failure need to be established.
Wider implications of the findings: This case report expanded the spectrum of *WEE2* gene mutations responsible for human fertilization failure and provided genetic evidence for fertilization failure.
Trial registration number: None

P-501 Model based on maternal age to determine the optimal number of blastocysts required to retrieve at least one euploid embryo

M. Ferrández Rives[1], M. Enciso[1], L. Ortega[2], Y. Galiana[2], B. Rodriguez-Estrada[1], I. Jurado[1], M. Berruezo[1], A.B. Climent[3], J. Sarasa[1], J. Aizpurua[4]

[1]iGLS, Genetic Laboratory, Alicante, Spain ;
[2]IVF Spain, Embryology department, Alicante, Spain ;
[3]IVF Spain, Statistics department, Alicante, Spain ;
[4]IVF Spain, Gynecology, Alicante, Spain

Study question: Is it possible to anticipate the number of blastocysts and/or cycles needed to retrieve at least one euploid embryo based on maternal age?

Summary answer: The probability of finding at least one euploid embryo can be estimated based on age and the number of blastocysts available.

What is known already: Aneuploidy in human preimplantation embryos plays an important role in assisted reproduction treatments, being one of the main reasons for implantation failures and miscarriages. The impact of female age on euploidy rate is well-known, as with advancing maternal age, the risk of chromosomal abnormalities in embryos increases. Although patients undergo preimplantation genetic testing because of the clinical significance of aneuploidy, unfortunately the retrieval of euploid embryos is not guaranteed and limited information about the probability of obtaining euploid embryos is available. The estimation of the probability of euploid embryo retrieval would enable effective treatment plans and accurate counselling of patients.

Study design, size, duration: This is a retrospective study including data from a total of 1048 cycles (476 own-oocyte cycles and 572 donor-oocyte cycles) undergoing PGT-A (Preimplantation Genetic Testing for Aneuploidy) at blastocyst stage between January 2017 and January 2020. A total of 3940 embryos were studied. Relationship between maternal age, total number of blastocysts biopsied and embryo euploidy was assessed.

Participants/materials, setting, methods: Patients referred for PGT-A analysis for a variety of reasons including advanced maternal age, recurrent implantation failure, recurrent miscarriage and male factor infertility (aged 24-54 years) were included in the study. All embryos were cultured to blastocyst stage, biopsied and PGT-A-tested for all 24 chromosomes using Next Generation Sequencing (Illumina, VeriSeq protocol). Outcome measures were: number of blastocysts (BT), euploidy rate (ER) and the probability of finding at least one euploid embryo for transfer (POE).

Main results and the role of chance: Our results show a negative influence of female age on euploidy rate and the number of blastocysts per cycle ($p<0.001$). Moreover, the probability of finding at least one transferable euploid embryo (POE) is strongly associated to age and the number of blastocysts available. POE is highest in egg-donation cycles (96%) and it reached 100% in cohorts of >3 blastocysts. Nevertheless, POE decreases with age (approximated by the following quadratic function: $y = -0.003x^2 + 0.1785x - 1.6825$, $R^2=0.91$) and this decline is very sharp after 40 years old.

In women <35 years old, the mean number of blastocysts per cycle (4.3) guarantees one euploid embryo in 96% of cases. Meanwhile, women aged 35-40 and over 40 produce 3 and 2.2 blastocysts per cycle, respectively. In these groups, the availability of at least one euploid embryo is guaranteed in 65% and 28% of cycles, respectively. POE decline with age can be mitigated by the banking of embryos from various cycles. This embryo banking strategy significantly increases the likelihood of euploid embryo transfer in advanced maternal age groups. The presence of one euploid embryo in the group of patients over 40 studied can be guaranteed in cohorts of >7 blastocysts (~3-4 cycles).

Limitations, reasons for caution: This model can serve as a valuable tool to improve assisted reproduction treatment results, however, other variables such as the number of oocytes retrieved, sperm quality parameters or AMH levels may also need to be considered as potential factors with influence in the model.

Wider implications of the findings: Our results confirm the well-known negative effect of female age on embryo euploidy. We have identified a model based on maternal age able to predict the likelihood of euploid embryo transfer. This model can help doctors to plan their treatments and counsel patients about their options of success.

Trial registration number: not applicable

P-502 Low-degree mosaicism profiles do not provide clinically useful predictive values: interim results from the first multicenter prospective non-selection study on the transfer of mosaic embryos

A. Capalbo[1], M. Poli[1], D. Cimadomo[2], F. Benini[3], C. Patassini[1], C. Rubio[4], E. Albani[5], L. Sacchi[5], L. Rienzi[2], C. Livi[3], P.E. Levi-Setti[5], M.U. Filippo[2], C. Simón[1,4,6,7]

[1]Igenomix, Reproductive Genetics, Marostica, Italy ;
[2]Genera, Reproductive Medicine, Rome, Italy ;
[3]ART Center DEMETRA, Reproductive Medicine, Florence, Italy ;
[4]Igenomix Foundation, Reproductive Genentics, Valencia, Spain ;
[5]Humanitas Clinical and Research Center- IRCCS, Division of Gynecology and Reproductive Medicine- Humanitas Fertility Center, Milan, Italy ;
[6]Valencia University and INCLIVA, Department of Obstetrics and Gynecology, Valencia, Spain ;
[7]Harvard School of Medicine- Harvard University- MA- USA, Department of Obstetrics and Gynecology, Boston, U.S.A.

Study question: What is the impact on clinical outcome of putative low-grade mosaicism diagnosis?

Summary answer: Blastocysts diagnosed with putative low-grade mosaicism produce comparable clinical outcomes to uniform euploid blastocysts in terms of sustained implantation and miscarriage rates.

What is known already: Highly sensitive NGS-based technologies allow precise discrimination of subtle chromosomal copy number variations (CNVs) in multicellular biopsies.These alterations are often interpreted as evidence of an embryo's chromosomal mosaicism. Because of the unknown clinical impact of mosaicism, uniformly euploid embryos are prioritized for transfer, whilst putative mosaic ones are given low priority or even discarded. Presently, transfer outcomes of putative mosaic embryos have only been compared retrospectively in selected subpopulations of patients that failed to get pregnant with previous euploid embryos. This prospective non-selection study was designed to avoid/minimize confounding factors and provide robust evidence on putative mosaicism clinical predictive values.

Study design, size, duration: This is an interim analysis of a multicenter prospective non-selection study of consecutive cases performed at five IVF clinics between Sept2018 and July2019. Trophectoderm biopsies showing intermediate chromosome CNVs consistent with low mosaicism (20-50%) were blindly reported as euploid. The presence of low-grade alterations did not influence the embryo selection process, thus allowing an unbiased comparison of clinical outcomes between fully euploid and putative mosaic embryos. Ethical committee approvals were obtained at each site.

Participants/materials, setting, methods: Consecutive patients (female age 35-44) undergoing homologous IVF cycles with blastocyst-stage PGT-A and single frozen euploid embryo transfer (SEET) were enrolled. Main exclusion condition was blastocyst of the worst morphological class. Sustained implantation rate (>20weeks) was the primary outcome measure. A sample size of 878 SEET was planned (beta=0.80; alfa=0.05) assuming 47% sustained implantation rate in the control group and 12% variation. This sample-size is also powered to detect meaningful differences in the miscarriage rate.

Main results and the role of chance: This study evaluates transfer outcomes of 368 SEET: 197 from uniform euploid embryo group (group A), 94 from putative very-low mosaic (20-30%, group B) and 77 from the putative low mosaic group (30-50%; group C). Positive pregnancy test rate was 58.4% (95%CI=51.2%-65.3%), 60.6% (95%CI=50.0%-70.6%) and 58.4% (95%CI=46.6%-69.6%) for group A, B and C, respectively (P=NS). Biochemical pregnancy loss rate was 9.6% (95%CI=4.9%-16.5%),10.5% (95%CI=4.0%-21.5%) and 13.3% (95%CI=5.0%-26.8%) for A, B and C respectively (P=NS). Miscarriage rate was also not significantly different across groups: 12.5% (95%CI=6.8%-20.4%), 13.7% (95%CI=5.7%-26.3%) and 12.8% (95%CI=4.3%-27.4%) for A, B and C, respectively (P=NS). Sustained implantation rate was also similar: 46.2% (95%CI=39.1%-53.4%), 48,8% (95%CI=36.4%-57.4%) and 44.2% (95%CI=32.8%-55.9%), for A, B and C respectively (P=NS). Multivariate logistic regression analysis including main and potentially relevant patient and cycle factors showed slower embryo development (embryo developed to blastocysts on day 7) as the only covariate associated with sustained implantation outcome (R=0.19; 95%CI=0.05-0.7). In the multivariate analysis, after adjusting for confounding factors, the presence of a PGT-A analysis consistent with mosaicism was not significantly associated with either primary or secondary outcome measures (OR=1.001; 95%CI=0.61-1.06). When groups B and C were combined, the lack of association persisted.

Limitations, reasons for caution: Although current interim data are sufficiently powered to exclude clinically relevant differences between study groups, the planned sample size isn't yet fully met. Study's main limitation consists in the lack of cytogenetic analysis follow-up of putative mosaic embryos pregnancies. However, indication for invasive prenatal testing couldn't be justified by evidence.

Wider implications of the findings: This is the largest prospective non-selection study evaluating the outcome of putative mosaic embryo transfers. If confirmed, these results demonstrate that intermediate CNVs consistent with low-grade mosaicism don't provide any clinically useful diagnostic criteria for defining embryonic reproductive competence. Re-assessment of aneuploidy categorization and embryo selection schemes will be required.

Trial registration number: NCT03673592

P-503 Assessing egg utilization efficiency from the outcome of Preimplantation Genetic Testing cycles

W. Wu[1], X. Yang[2]

[1]First Affiliated Hospital of Nanjing Medical University, Reproductive Medical Center, Nanjing, China ;
[2]Nanjing Medical Univesity, First Clinical Medical College, Nanjing, China

Study question: In patients using preimplantation genetic testing (PGT), the average number of eggs needed to obtain 1, 2, and ≥3 euploid/balanced embryos was calculated for populations with different characteristics.
Summary answer: Couples with one or both reciprocal translocation carriers need more eggs to obtain the same number of euploid/balanced embryos,especially those with general balanced translocation carriers.
What is known already: Chromosome rearrangement can significantly affect fertility and increase the risk of miscarriage. Although there are theoretical models for the probability of normal gamete formation among balanced translocation carriers, more clinical data are needed to support the average number of eggs required to obtain the expected number of euploid/balanced embryos during ovarian stimulation process.
Study design, size, duration: From June 2011 to July 2019, 1,659 PGT retrieval cycles were collected.There were 368 cycles in PGT for aneuploidies(PGT-A group), 105 cycles in PGT for monogenetic(PGT-M group) and 1186 cycles in PGT for structural rearrangements which including 829 cycles of general translocation group and 357 cycles of Robertson translocations group.
Participants/materials, setting, methods: All patients were treated with appropriate ovarian stimulation protocol.When the diameter of the dominant follicles reached about 18mm, human chorionic gonadotropin (HCG) was injected and ovum extraction was conducted under the guidance of transvaginal ultrasound 36 hours later.

Embryo testing: blastocyst or blastomere biopsies are performed using array-based comparative genomic hybridization (aCGH) or next-generation sequencing (NGS).
Main results and the role of chance: The average number of eggs needed to obtain1, 2 and ≥3 euploid/balanced embryos in the PGT-A group was 7.65, 10.38, and 12.95, respectively; the average age of the woman was 33.83 ± 4.62, and the average age of the man was 35.40 ± 5.67. The average number of eggs needed to obtain1, 2 and ≥3 euploid/balanced embryos in the PGT-M group was 7.4, 10.77, and 14.33, respectively; the average age of the woman was 31.45 ± 4.04, and the average age of the man was 32.53 ± 4.87. The average number of eggs needed to obtain 1, 2 and ≥3 euploid/balanced embryos in the PGT-SR general translocation group was 11.1, 13.32, and 15.59, respectively; the average age of the woman was 28.97 ± 3.86, and the average age of the man was 30.32 ± 4.95. The average number of eggs needed to obtain 1, 2 and ≥3 euploid/balanced embryos in the Robertson translocations group was 10.01, 12.49, and 15.32, respectively; the average age of the woman was 29.30 ± 4.04, and the average age of the man was 30.84 ± 4.81.
Limitations, reasons for caution: Patients using PGT-M are first tested for a single gene of the relevant disease.Aneuploidy screening will not be continued if they carried the pathogenic gene,which causing the average number of eggs needed to obtain euploid/balanced embryos increased.The number of patients included in the PGT-M group was relatively small.
Wider implications of the findings: The study provides clinical data on the expected number of eggs required to obtain euploid/balanced embryos in different patient populations with different characteristics, and provides a reference for clinicians to determine the optimal ovarian stimulation protocol for patients.
Trial registration number: without

P-504 Increased Body Mass Index and Its Impact on Blastocyst Ploidy Status

A. El-Damen[1], I. Elkhatib[1], A. Bayram[1], A. Arnanz[1], A. Abdala[1], N. Nobrega[1], S. Samir[2], L. Melado[3], B. Lawrenz[3], N. De Munck[1], H. Fatemi[3]

[1]IVI Middle East Fertility Clinic, IVF Laboratory, Abu Dhabi, United Arab Emirates ;
[2]IVI Middle East Fertility Clinic, Administration, Abu Dhabi, United Arab Emirates ;
[3]IVI Middle East Fertility Clinic, Clinical Department, Abu Dhabi, United Arab Emirates

Study question: Does increased Body Mass Index (BMI) affect blastocyst ploidy and cycle outcomes in IVF/ICSI patients with Preimplantation Genetic Testing for Aneuploidy (PGT-A)?
Summary answer: Increased BMI has no correlation with blastocyst ploidy; but decreases the number of mature oocytes per large follicles and per total doses of stimulation drugs.
What is known already: World Health Organization (WHO) identified obesity as a global epidemic as its prevalence has tripled since 1975. It is a major risk factor for many diseases including infertility, with a prevalence of 71% of the female Middle Eastern population being affected. Obesity has a detrimental effect on female fertility by causing hypothalamic-pituitary dysfunction which may lead to anovulation and impaired follicular development. Increased BMI has a negative prognosis factor for cycle cancellation, the number of oocytes collected, the number of embryos available, clinical pregnancy rate and abortion rate which may be due to both impaired oocyte quality and uterine function.
Study design, size, duration: A retrospective cohort study including 1115 fresh IVF/ICSI cycles from 807 patient was conducted in a single private fertility clinic in Abu Dhabi, UAE between March 2017 and December 2018. According to WHO, patients were stratified as: underweight, normal, pre-obese, obese I, obese II and obese III when they have a BMI of: <18.50, 18.50–24.99, 25.00–29.99, 30.00–34.99, 35.00-39.9 and ≥40 kg/m^2, respectively. ANOVA test was used to compare means of different parameters.
Participants/materials, setting, methods: Females were ≥18 years old who underwent fresh IVF/ICSI treatment with PGT-A. Routine work included ovarian stimulation using different types of protocols. Fresh oocytes were inseminated either by IVF or ICSI and the resulted fertilized oocytes were cultured till blastocysts were biopsied on day 5, 6 and 7 of development and tested for PGT-A using NGS. blastocysts were vitrified and euploid ones were warmed and transferred in a subsequent natural or hormonal replaced cycle.
Main results and the role of chance: We stratified our population regarding BMI into the following groups: Normal, underweight, preobese, obese I, obese II and obese III with the following percentages: 35.8%, 1.8%, 37.9%, 18.8%, 5% and 0.7% (n= 400, 20, 422, 209, 56 and 8) respectively. Our study demonstrated no significant difference between the groups regarding number of stimulation days (p=0.074), oocytes retrieved (p=0.66), mature oocytes (p=0.93), fertilized oocytes (p=0.72), biopsied (p=0.21) and euploid blastocysts (p=0.22). The same non-significant difference was also reported regarding fertilization and euploidy rates (p=0.26 and p=0.19). Interestingly, negative correlations were found between BMI and (i) the number of mature oocytes per follicles >11mm (p= 0.044), (ii) aspirated oocytes per total stimulation doses (p= 0.020) and (iii) mature oocytes per total stimulation doses (p= 0.029). Correcting for BMI as a confounder and applying multivariate regression and Poisson models resulted in a negative correlation between euploidy rate and age and a positive correlation between euploidy rate and the number of mature oocytes (both p<0.001).

Applying a multivariate regression model and correcting for age and number of mature oocytes collected revealed that increased BMI did not have any impact on implantation (51.2% vs 50.8%p=0.079) or clinical pregnancy rates(47.4% vs 46.7%, p= 0.092) respectively.
Limitations, reasons for caution: Our results were based on retrospective data from a single fertility clinic, which does not permit to control for various factors leading to the selection of a stimulation protocol by a physician for each patient.
Wider implications of the findings: Although increased BMI doesn't affect euploidy rate or cycle outcomes of patients undergoing IVF/ICSI with PGT-A, obese patients need to be administered more stimulation doses for them to have the same number of mature oocytes as the normal weight patients, which decreases the cost-effectiveness of their treatment cycles.
Trial registration number: NA

P-505 BCORL1, USP9x and POF1B copy number variation in premature ovarian failure: A Preliminary study

C. Furtado[1], F. Gennaro[2], G. Kogure[3], R. Ferriani[4], R. Reis[3]

[1]Federal University of Ceara, Drug Research and Development Center-Postgraduate Program in Medical and Surgical Sciences, Fortaleza, Brazil ;
[2]Ribeirao Preto Medical School- University of Sao Paulo, Obstetrics and Gynaecology, Ribeirão Preto, Brazil ;
[3]Ribeirao Preto Medical School- University of Sao Paulo, Obstetrics and Gynaecology, Ribeirao Preto, Brazil ;

[4]Ribeirao Preto Medical School- University of Sao Paulo-, Obstetrics and Gynaecology, Ribeirao Preto, Brazil

Study question: Copy number variation (CNV) in X-chromosome linked genes are related to idiopathic premature ovarian insufficiency (POI)?
Summary answer: POI presented alteration in the number of copies for the X-linked genes *USP9X* and *POF1B*.
What is known already: X-linked genes are strongly associated with ovarian function and have several POI candidate genes. Both amplifications and deletions can alter the level of expression of clusters of genes associated with POI, including genes involved in pairing and segregation such as *POF1B* and *USP9X* and in response to apoptosis *BCORL1*. Consequently, those alterations may promote changes in the XCI pattern, such as nonrandom (skewed) X inactivation in female genome and maybe an important feature of POI.
Study design, size, duration: Cross-sectional study, in which 37 non-syndromic POI women were included.
Participants/materials, setting, methods: POI (FSH>40 IU/l) with 46,XX karyotype participated in this study. Age, body mass index (BMI) were analyzed. The CNVs in the *BCORL1*, *USP9X* and *POF1B* was measured by quantitative real time PCR and the reference genes were the *HPRT1* (x-linked) and the *MFN2* (autossomal). A control DNA (male and female) was used in the comparisons. XCI was based in the Human Androgen Receptor (HUMARA) and X-linked retinitis pigmentosa 2 (*RP2*) assays.
Main results and the role of chance: The mean age was 32.10 ± 6.81 and BMI was 25.41 ± 4.64. Most POI women presented number of copies (> 0.75) close to the female control (46, XX) for the *BCORL1* (97.2%), *USP9X* (55.6%) and *POF1B* (58.3%). Reduced number of copies similar to the male control (<0.75) was observed for the *USP9X* (44.4%) and *POF1B* (41.17%). Skewed XCI (≥75%) was observed in 24,32% (n=9) POI. From those POI with copy number alterations only 10% presented skewed XCI.
Limitations, reasons for caution: Limited number of cases of the study group. An increase of sample size is recommended to make the data more robust and confirm the results.
Wider implications of the findings: Alterations in X-linked genes and skewed XCI was observed in POI women, a genetic condition that is frequently observed in ovarian dysfunctions, suggesting a possible role in POI etiology.
Trial registration number: 13305/2012

P-506 Determining copy number variation in SMN1 using a highly multiplexed Next Generation Sequencing panel

A. Harris[1], G. Del Mistro[1], T. Fahland[1], H. Xie[1], J. Bishop[1], J. Veitch[2], V. Mozhayskiy[1]

[1]Thermo Fisher Scientific, Clinical Next Generation Sequencing, Carlsbad- CA, U.S.A. ; [2]Thermo Fisher Scientific, Clinical Next Generation Sequencing, South San Francisco- CA, U.S.A.

Study question: Can a Next Generation Sequencing (NGS) panel be used to accurately assess samples for copy number variation (CNV) in genes that have high homology such as SMN1 and SMN2?
Summary answer: We were able to characterize test samples for functional SMN1 copy number with 97.8% accuracy and SMN2 copy number with 95.6% accuracy (n=91).
What is known already: Loss of SMN1 activity is a cause of spinal muscular atrophy (SMA). SMN1 and SMN2 are highly homologous genes that are difficult to distinguish on NGS platforms due to relatively short read lengths. Typically the copy number determination of these genes is assessed with other technologies such as Multiplexed Ligation Probe Assays (MLPA) and microarray.
Study design, size, duration: We tested 91 gDNA samples from Coriell cell lines with functional SMN1/2 copy number status known from microarray. The samples were tested as part of a characterization of the NGS panel in development. Results needed to meet pre-defined quality control criteria to be included in the assessment.
Participants/materials, setting, methods: Samples were amplified using a two-pool primer panel comprised of approximately 14,000 PCR primer pairs targeting coding sequences and known intronic variants in 420 genes implicated in recessive autosomal and X-linked diseases. The resulting DNA barcoded libraries were sequenced in a multiplex of 16 per Ion 540™ Chip using the Ion S5™ Sequencing System.
Main results and the role of chance: We used an *in silico* baseline derived from amplicon representation from normal samples and a custom CNV calling pipeline to determine both i) the mean copy numbers from amplicons with 100% identity between homologous portions of the genes of interest and ii) the homolog-specific copy numbers for amplicons with distinguishing sequence variants between the primers or sequence variants under the primers which enriched amplification in one member of a gene pair. Using this information and orthogonal microarray results taken as "truth," we were able to obtain SMN1 carrier status with 100% sensitivity (n=4) and 98.9% specificity (n=87). Follow up will be needed from individual labs employing our test to determine reproducibility with their own samples of interest.
For Research Use Only. Not for use in diagnostic procedures.**Limitations, reasons for caution:** We tested our method on a limited set of gDNA samples isolated from cell lines. Factors that could compromise performance for other samples include amplification and sequencing variation not accommodated by our CNV baseline and SNPs falling under primers resulting in decreased amplification efficiency that mimics a copy number loss.
Wider implications of the findings: Our panel design and analysis methods enable the determination of SMN1/2 copy number as part of a much broader NGS assay of hundreds of genes responsible for inherited diseases, enabling the development of more convenient future tools.
Trial registration number: not applicable

P-507 DNA detection in the blastocoelic fluid (BF) from expanded blastocysts generated by conventional IVF cycles: clinical implications

C. Albanese[1], M.C. Magli[1], S. Azzena[1], M. Piiffanelli[1], B. Balicchia[1], C. Tabanelli[1], L. Gianaroli[1]

[1]S.I.S.Me.R., Reproductive Medicine Unit, Bologna, Italy

Study question: Does the presence or absence of DNA in the BF correlate with blastocyst viability?
Summary answer: After whole genome amplification (WGA) of BFs, failure to detect DNA correlates with significantly higher implantation rates when compared to blastocysts with positive DNA amplification.
What is known already: The presence of DNA in the BF could represent a strategy towards a euploid condition through the extrusion of aneuploid cells into the blastocoelic cavity. This event could not occur at a measurable level when the blastocyst is fully or prominently euploid, whereas positive BF DNA amplification could indicate a blastocyst with aneuploid cells trying to correct a defective chromosome condition. Especially in the case of low to moderate level of mosaicism, the capacity of extruding the majority of abnormal cells into the BF would preserve the blastocyst potential of further development after transfer.
Study design, size, duration: This prospective cohort study included 184 conventional IVF cycles performed from January 2016 to October 2019. In 91 cycles (Study-group), the BF was collected from expanded blastocysts before vitrification and stored at -80°C. The control-group included 93 cycles, whose blastocysts were vitrified without blastocentesis. Single blastocysts were transferred by selection according to morphology. BFs were later submitted to WGA by operators blinded to the clinical outcome. WGA results were then related to implantation.
Participants/materials, setting, methods: Maternal age and indications to IVF were comparable between the two groups. The same protocol of vitrification was applied and only cycles with expanded blastocysts of high grade were included in the study. Amplification after WGA was evaluated by loading an aliquot of the amplified product onto a 1.5% agarose gel. A clinical pregnancy rate was defined by the presence of fetal heart-beat. The ongoing pregnancy rate was a pregnancy ongoing after 12 week gestation.
Main results and the role of chance: The clinical outcome was comparable in the study-group and control-group with similar clinical pregnancy rates (51 and 55% respectively) and ongoing pregnancy rates (47 and 47% respectively) suggesting that the procedure of blastocentesis per se had no impact on blastocyst viability. When the study-group was subdivided according to WGA results, we had 55 cases where BF failed to amplify (sub-group with failed BF-WGA) and 36 with positive amplification (sub-group with positive BF-WGA). Maternal age was 36.4±3.2 and 35.5±3.5 years respectively. When looking at the transfer outcome in the two sub-groups, the clinical pregnancy rate was significantly higher in the sub-group with failed BF-WGA (75%) when compared to the

sub-group with positive BF-WGA (25%, P<0.001). The ongoing pregnancy rate showed the same trend and was 69% and 11% respectively (P<0.001).
Limitations, reasons for caution: The study is a prospective cohort study, but not prospectively randomized.
Wider implications of the findings: Failure to detect DNA after BF amplification could represent an additional criterion to select viable embryos for transfer in conventional IVF cycles. If confirmed in a prospective randomized study, this approach would improve the clinical outcome of vitrified blastocysts by using a simple, non-invasive and moderately expensive approach.
Trial registration number: Not applicable

P-508 A pilot study to identify Single Nucleotide Variants (SNVs) as predictors of oocyte/embryo quality in fertile women undergoing Preimplantation Genetic Testing for Monogenic Disorders (PGT-M)

G. Kakourou[1], T. Mamas[1], C. Vrettou[1], E. Tsorva[2], S. Zacharia[2], C. Oraiopoulou[3], E. Chatzi[4], E. Kourkouni[5], G. Kourlaba[5], D. Christopikou[2], M. Mastrominas[2], M. Moysidou[3], A. Chatziparasidou[3], I. Georgiou[4], J. Traeger-Synodinos[1]

[1]National & Kapodistrian University of Athens, Department of Medical Genetics, Athens, Greece ;
[2]Embryogenesis, Assisted Reproduction Unit, Athens, Greece ;
[3]Embryolab SA, IVF Unit, Thessaloniki, Greece ;
[4]Genetics and IVF Unit- Faculty of Medicine- University of Ioannina, Department of Obstetrics and Gynecology, Ioannina, Greece ;
[5]Center for Clinical Epidemiology and Outcomes Research, Cleo, Athens, Greece

Study question: Are SNVs in 26 selected genes potentially involved in preimplantation development, associated with oocyte and embryo quality in fertile women undergoing PGT-M?
Summary answer: SNVs in 10/26 studied genes revealed significant associations with oocyte number, fertilization rate and blastocyst formation in fertile women undergoing PGT-M.
What is known already: Genetic profiling of prospective parents has been one of the approaches for identifying biomarkers of IVF success. Specific genotypes have been associated with IVF failure, gamete/embryo quality etc., with most studies focusing on infertile populations. Recent studies identified SNVs (rs1801133, rs1801131 in *MTHFR*, and rs2305957 on chromosome 4 linked to *INTU, SLC25A31, HSPA4L, PLK4, MFSD8, LARP1B* and *PGRMC2*) associated with embryo quality or chromosomal status in infertile women. Additionally, preimplantation development may be influenced by pathways involved in follicle development, meiosis, mitosis and DNA repair, for which many other genes have been implicated in human and animal studies.
Study design, size, duration: DNA from fertile prospective mothers was genotyped using exonic NGS (Qiaseq™ Targeted Custom Panel, Miseq) for 18 genes (*AIRE-AMH-AURKA-AURKB-AURKC-FSHR-HSPA4L-HUWE1-INTU-KHDC3L-LARP1B-MFSD8-MTHFR-PGRMC2-PLK4-SENP7-SLC25A31-WBP1*) and 9 selected SNVs in a further 8 genes: rs175080(*MLH3*), rs1799963(*F2*), rs6025(*F5*), rs5918(*ITGB3*), rs5985(*F13A1*), rs1805087(*MTR*), rs1801394(*MTRR*), rs28756992(*MLH3*) and rs2305957(*HSPA4L*). The study (September 2017-2019), approved by the University of Athens Bioethics Committee and National Authority of Assisted Reproduction, involved PGT-M cycles performed between 2013-2019. Statistical analysis was performed by the Center for Clinical Epidemiology and Outcomes Research.
Participants/materials, setting, methods: The study was performed at the UoA Laboratory of Medical Genetics, and focused on fertile couples undergoing PGT-M. NGS was performed for 85 women undergoing 107 PGT-M-cycles. The following details were collected: maternal age, stimulation protocol, number of oocytes collected/fertilized, blastocyst formation. Maternal genotypes were analysed in association with number of oocytes collected (No.O), fertilization rate (FR), percentage of blastocysts developed per MII oocyte (BL/MII) and percentage of blastocysts per 2PN embryo (BL/2PN).
Main results and the role of chance: A 20x coverage was achieved in all exons and SNV regions investigated, identifying 121 variants. Using STATA SE, v.13, to test for normality and associations between continuous variables and different genotypes, significant associations (p<0.05) were detected for each of the studied parameters: 1) No.O: lower for minor allele (MA) of rs564533 (*KHDC3L*), higher for TT vs. CC/TT genotype of rs7616677 (*SENP7*) and lower for AA vs. GA/GG genotype of rs939443 (*SENP7*). 2) FR: lower for MAs of rs1059476 and rs2241909 of *AURKB* and higher for MAs of rs61736572 and rs61736575 of *AMH*, higher for MA of rs56043017 of *PLK4*. 3) BL/MII: higher for MA of rs1801133 of *MTHFR*, lower for MAs of rs7616677 and rs939443 of *SENP7* and higher for MA of rs2433031 of *SENP7*. 4) BL/2PN: lower for MA of rs35518193 of *HSPA4L*, higher for MA of rs2433031 of *SENP7*. No significant associations were revealed for rs1801131 and rs2305957. Where comparisons were possible, these findings remained significant independent of age and stimulation protocol. Additional associations were revealed for rs3746964, rs878081, rs1800521 (*AIRE*), rs10407022 (*AMH*) and rs175080 (*MLH3*) when stratifying for age and stimulation protocol.
Limitations, reasons for caution: The main limitations of the study include its retrospective nature, whereby IVF practices have changed considerably over the period of the study (2013-2019). The polymorphisms identified with likely significant impact, require prospective validation in other PGT-M cycles, as well as on embryo samples of variable quality.
Wider implications of the findings: Infertility is a complex condition. Identifying prognostic factors in ART patients is confounded by multiple variables (parental age, stimulation and fertilization protocols, embryo transfer stage/conditions etc.). PGT-M facilitates investigation of a fertile ART population, minimizing many confounding variables, and potentially facilitating the identification of genomic biomarkers predictive of gamete/embryo quality.
Trial registration number: This study was supported by a State Scholarships Foundation (IKY) for post-doctoral research and co-funded by national grants and the European union (ESPA 2014-2020). Trial registration number not applicable

P-509 Applied machine learning based on time-lapse technology and reproductive history data designed for pre-implantation embryo ploidy prediction.

S. Davies[1], D. Christopikou[1], N. Kallioras[2], P. Tsakalis[2], E. Tsorva[1], S. Zaxaria[1], K. Economou[1], R. Najdecki[3], E. Timotheou[3], P. Tatsi[3], T. Chartomatsidou[3], C. Papanikopoulos[1], N. Lagaros[2], M. Mastrominas[1], E. Papanikolaou[3]

[1]c/o Embryogenesis-Private Centre for Reproductive Studies, embryology, Maroussi- Athens, Greece ;
[2]Institute of Structural Analysis & Antiseismic Research- National Technical University of Athens, School of Civil Engineering, Athens, Greece ;
[3]Assisting Nature- Private IVF Unit, Ob/gyn, Thessaloniki, Greece

Study question: To determine whether Artificial Intelligence (AI) algorithms based on time-lapse (TLM) morphokinetic parameters and reproductive history can be used to predict embryo ploidy status.
Summary answer: Cleavage time intervals from 5-6-7-8-9-cell stage to time of morulation, along with maternal age and PGT indication enhances the prediction of blastocyst ploidy.
What is known already: Time-lapse technology allows continuous monitoring of embryo development and has assisted in progression towards automated and objective systems to assess the embryos and improve IVF treatment. Artificial intelligence technology, based on data and pattern exploitation, has been reported as a tool to identify embryo viability and predict IVF outcome. However, studies reporting an association between morphokinetic parameters and embryo ploidy status are controversial and do not support the predictive value of time-lapse analysis for preimplantation genetic testing of embryo ploidy thus far.
Study design, size, duration: Double blind, retrospective longitudinal cohort study. Embryo morphokinetic parameters of 383 blastocysts were collected from 193 patients undergoing preimplantation genetic testing for aneuploidy (PGT-A) and structural rearrangements (PGT-SR) between November 2017 and December 2019 from two independent IVF clinics.
Participants/materials, setting, methods: The morphokinetic parameters of blastocysts, subject to PGT-A/PGT-SR analysis with next generation sequencing (NGS), were used to validate and test for ploidy prediction through a set of machine learning classifier algorithms. Data augmentation and pre-processing methods were applied on the sample to ensure unbiased results and optimal feature selection. Validation and testing were performed through a stratified five-fold split. The efficacy of ploidy prediction was quantified using ROC curves, AUC and confusion matrices.

Main results and the role of chance: After exhaustive feature selection via methods like Pearson Correlation and Decision Tree algorithms the following morphokinetic and reproductive history features were shown to contribute the most to predicting euploidy: tPNf, t4, t7-tPNf, tM-t5, tM-t6, tM-t7, tM-t8, tM-t9, maternal age and indication of PGT (PGTSR vs PGTA for recurrent miscarriages, advanced maternal age or severe male factor). A model was then created based on those parameters which showed a significant AUC=0.70 from the ROC curve analysis. The mean accuracy throughout the 5 validation folds was measured at 0.68 and the mean precision at 0.73. Out of 383 samples (150 euploid/ 233 aneuploid) the classification results, obtained from confusion matrices were: 157 true positives (TP), 76 false positives (FP), 96 true negatives (TN), 54 false negatives (FN). The features used can be sorted according to their correlation strength (higher to lower) as follows: PGT indication, Maternal Age, tM-t9, tM-t7, tM-t8, tM-t6, tM-t5, t7-tPNf, tPNf and t4. It is observed that lower values of maternal age, tM-t9, tM-t7, tM-t8, tM-t6, tM-t5 and tPNf correspond to higher probability for euploidy while the opposite stands for t7-tPNf and t4.
Limitations, reasons for caution: The main limitation of the current study is the small sample size. Machine learning algorithms provide better results and generalize efficiently when sufficient data is provided.
Wider implications of the findings: Machine learning model depicts specific cleavage time intervals, maternal age and PGT indication as significant predictors of embryo ploidy. This model cannot substitute pre-implantation genetic testing for aneuploidy but can potentially be useful during the prioritization of embryos for transfer in cases where PGTA/PGTSR is not performed.
Trial registration number: non applicable

P-510 Detailed investigation into the mosaic embryo karyotypes: multicenter data from 2280 trophectoderm biopsies obtained during preimplantation genetic testing cycles in IVF

F. Fiorentino[1], F. Spinella[1], A. Biricik[1], A. Victor[2], F. Barnes[2], C. Zouves[2], A. Ruberti[3], E. Cursio[3], M. Sofia[4], L. Corti[4], V. Bianchi[5], P. Viganò[4], M.G. Minasi[3], G. Ermanno[3], M. Viotti[2]

[1]Genoma Group srl, Molecular Genetics Laboratories, Rome, Italy ;
[2]Zouves Foundation for Reproductive Medicine, Zouves Foundation, Foster City, U.S.A. ;
[3]European Hospital-, Centre For Reproductive Medicine., Rome, Italy ;
[4]San Raffaele Scientific Institute, U.O. Obstetrics and Gynecology, Milan, Italy ;
[5]Future for Family, Future for Family, Bologna, Italy

Study question: Which is the prevalent type of mosaicism affecting human mosaic embryos?
Summary answer: Single whole chromosome aneuploidy is the most prevalent form of mosaicism in preimplantation embryos with a majority involving gains.
What is known already: Chromosomal mosaic embryos are characterized by the presence of chromosomally different cell lines within the same embryo. Mosaicism may involve whole-chromosome, segmental (or partial), complex or a combination of such aneuploidies. We previously demonstrated that the reproductive potential of mosaic embryos is affected by the complexity of and the number of aneuploid cells present in trophectoderm (TE) biopsy. We also observed that the mosaicism involved specific chromosomes and that single (sub-chromosomal or whole-chromosome) aneuploidy was the prevalent type of chromosomal mosaicism. However, our study involved a limited number of mosaic embryos and data available were insufficient to test this hypothesis.
Study design, size, duration: This is a large-scale multicenter study on mosaic embryos to examine the patterns and prevalence of chromosome specific mosaicism in TE samples. The cohort consisted of 2280 consecutive mosaic embryos collected between May2016-May2019. All embryos were cultured to blastocyst stage; TE biopsy was performed on Day-5 or Day6/7of development. TE biopsies underwent comprehensive chromosome screening utilizing validated next generation sequencing (NGS). TE biopsies were classified as mosaic if they had 20%-80% abnormal cells.
Participants/materials, setting, methods: Mosaic embryos composed of mosaic chromosomes only were analyzed. Mosaicism was tabulated per chromosome, and chromosomal constitution and incidence of different type of mosaic embryos were also analyzed. For statistical analysis mosaic embryos were divided in three groups: whole-chromosome, segmental and mixed mosaicism. In addition, whole-chromosome and segmental mosaicism were divided based on chromosomal constitution in single (monosomy or trisomy), double, and complex aneuploidy (more than two different aneuploidies) group.
Main results and the role of chance: A total of 4850 aneuploidies were detected, whole-chromosome (3547/4850;74%) occurred more frequently than segmental (1303/4850; 26%) mosaicism (P<0.01). The highest prevalence of whole-chromosome imbalance leading to aneuploidy was seen for chromosome 14, 18, 21, 22 and X, while for segmental mosaicism was seen for chromosomes 1,2,5, and 16. Mosaicism rates for these chromosomes did not statistically vary when stratified by maternal age. For whole-chromosome mosaicism, trisomy was significantly more frequent than monosomy (p<0.05) but for segmental mosaicism trisomy was less frequent (P<0005). Regarding the type of mosaic embryos, 53% (1209/2278) were found to be composed of whole chromosomes, 30% (694/2278) of structural aneuploidies and 17% (375/2278) contained both whole-chromosomal and structural aneuploidies. Of the whole-chromosome embryos, single aneuploidy was significantly more frequent than complex (53%, vs 28%;p<0.001), and double aneuploidy (53%, vs 19%;p<0.001). Similarly, when grouped based on structural abnormalities, single segmental aneuploidy was significantly more frequent than double (80% vs 16%; p<0.0001) or complex segmental aneuploidy (80% vs. 4%; p<0.001). Structural mosaic and whole-chromosome aneuploidy blastocysts with >50% aneuploid cells accounted for 11% and 14% of analysed embryos, respectively.
Limitations, reasons for caution: This study was retrospective, demonstrating the relative frequency of different type of mosaic embryos but not offering any direct insight into the clinical relevance of the findings. Additional clinical data must be obtained to evaluate the clinical implication of chromosomal mosaicism in mosaic embryo outcome.
Wider implications of the findings: Our findings reported the prevalence of the different kind of mosaicism in human blastocyst. Furthermore the study provides a detailed description of the prevalence, distribution and level of mosaicism for each chromosome involved in mosaicism. These results contribute to the understanding of the nature and origin of mosaic embryos.
Trial registration number: None

P-511 Whole genome insights into male infertility

A. Mantzouratou[1], L. Xanthopoulou[2], A. Mania[3], P. Serhal[4], J. Delhanty[5], S. SenGupta[5]

[1]Bournemouth University, Life and Environmental Sciences, Poole, United Kingdom ;
[2]Cooper Genomics, Laboratory Director, London, United Kingdom ;
[3]King's Fertility, The Fetal Medicine Research Institute, London, United Kingdom ;
[4]CRGH, The Center for Reproductive and Genetic Health, London, United Kingdom ;
[5]University College London, Institute for Women's Health- Preimplantation Genetics Group, London, United Kingdom

Study question: How is structural genetic variation associated with the causes and prognosis of poor sperm parameters and embryonic aneuploidy using a whole genome sequencing (WGS) approach.
Summary answer: Males with severe sperm abnormalities present with significantly higher deletion length and increased amount of CNVs throughout their genome that could link to embryonic aneuploidy.
What is known already: Poor sperm parameters are associated with embryonic aneuploidy and linked to various polymorphic sites concentrated in the Y chromosome and several individual genes. Structural genomic variations in individuals have been linked abnormal sperm parameters. So far studies have shown that male infertility is the result of a combination of several genetic and environmental interactions but in most cases the approach was targeted to specific areas of the genome with varied results. The impact of genetic variation on sperm parameters and embryonic aneuploidy is not yet fully established. Whole genome sequencing can provide a more complete genomic snapshot of these conditions.
Study design, size, duration: Couples presenting with repeated implantation failure and/or recurrent miscarriage that went through PGT-A (2008 - 2015) were invited to provide DNA samples for WGS.
Several selection criteria were applied to the test group: i) referred for PGT-A >3 AR failures, ii) Normal karyotypes, iii) Female age less than 37, iv) Embryonic instability in their subsequent PGT-A cycles. Results were analysed according to insemination method, sperm parameters and associated with the PGT-A embryo results.

Participants/materials, setting, methods: From the invited individuals - 118 consented to WGS analysis. From the consented individuals, 36 (18 couples) passed all the selection criteria. Within that cohort, there were 12 males needing ICSI and poor sperm parameters and 9 males with normal sperm parameters (IVF cohort) and DNA from these participants was sequenced.
WGS was performed with a short-read BGI platform. Variant calling and genomic data analysis were performed with gold standard bioinformatic pipelines.
Main results and the role of chance: Whole genome sequencing provides a global view of the human genome and is a step towards a personalized approach to healthcare.

This study has found that males with severe sperm abnormalities present with high DNA deletion length and increased amount of copy number variations compared to less severely affected individuals and to males with normal sperm parameters. Specifically, the ICSI group had significantly higher total CNV deletion length than the IVF group. In addition, the total number of CNV's was significantly higher in the males with severe conditions like azoospermia compared with the males that presented with poor sperm parameters like oligospermia. These variations were found in several areas of the genome as well as in the Y chromosome or genomic areas related to fertility.

Several polymorphic sites found in the same genomes could contribute to an accumulated risk that is linked to poor sperm parameters and embryonic aneuploidy. For example, in severely affected individuals, structural variations were found in several genes associated with spermatogenic failure like AURKC, CATSPER1, CEP19. Variations in genes associated with increased prostate cancer risk observed in some men with poor sperm parameters. This could explain the highly heterogeneous associations in genetics of male infertility.
Limitations, reasons for caution: Ethnicity bias presented in relation to the bioinformatic analysis as variation is flagged based on frequencies mainly focused in Caucasian populations, so this has been considered especially for the SNP analysis. For future work, a normal fertility control group with preimplantation embryo results would facilitate further research.
Wider implications of the findings: A complex picture of a polygenetic/multifactorial risk is arising for certain infertility types, the results from such targeted population studies can help in the identification and subsequently the prediction of genetic risk for heterogeneous conditions. This study adds to the prognostic value of WGS in male infertility and embryo aneuploidy.
Trial registration number: Not applicable

P-512 A review of 1,504 autologous embryos evaluated using a non-invasive platform for preimplantation genetic testing for aneuploidy at a private clinic

M. Olcha[1], M. Elzaky[1], M. Jaremko[1], Z. John[1], B. Hensinger[2], S. Lu[2]

[1]New Hope Fertility Center, Reproductive Endocrinology, New York, U.S.A. ;
[2]Yikon Genomics, Genomics, Lewes, U.S.A.

Study question: To determine if a non-invasive approach for preimplantation genetic testing for aneuploidy (PGT-A) can successfully assess embryo ploidy status at a large-scale private practice setting
Summary answer: Non-invasive PGT-A can identify ploidy status in a large cohort of embryos. Furthermore, this technology can also distinguish mosaic embryos from aneuploid or euploid ones.
What is known already: PGT-A has been widely used around the world to identify ploidy status of embryos. The knowledge obtained can help assist clinicians and patients with prioritization of embryos for transfer. The transfer of single euploid embryos has be shown to improve per cycle pregnancy rate, reduce miscarriage rate, and prevent transfer of embryos harboring certain genetic anomalies such as Down Syndrome. The primary modality for obtaining DNA from blastocysts has been trophectoderm biopsy, which requires removal of embryonic cells. Recently, the advent of non-invasive methods for PGT-A allow embryonic DNA within spent media to be amplified for ploidy analysis.
Study design, size, duration: Retrospective data analysis of 1,504 blastocysts, which were evaluated using non-invasive PGT-A from August 1st 2019 to December 30th 2019. All embryos evaluated using non-invasive PGT-A were included except patients using donor eggs.
Participants/materials, setting, methods: Patients electing to have their embryos undergo PGT-A were offered to utilize a validated non-invasive approach instead of conventional trophectoderm biopsy after appropriate informed consent. Spent media collected from embryos on day 5, 6, or 7 was processed on site with the Non-Invasive Chromosomal Screening (NICS) platform (Yikon Genomics, Lewes, DE). NICS uses multiple annealing and looping-based amplification cycles (MALBAC) for whole-genome amplification followed by next-generation sequencing to obtain ploidy information on all 24 chromosomes.
Main results and the role of chance: The average patient age was 36.6 years old. Of the 1,504 media samples tested with the NICS platform, 86 (5.7%) were initially found be inconclusive. 62 of the 86 inconclusive embryos were thawed for overnight culture and next day repeat media collection based on patient preference. The remaining 24 inconclusive were not resampled. All retested embryos resulted in usable ploidy data. The proportion of Euploid, Mosaic, and Aneuploid calls in the 1480 resulted embryos (original and retested) were 22.9%, 32.6%, and 44.5%, respectively. Of the 62 retested embryos, 35.4% were euploid, 29.0% were mosaic, and 35.5% were aneuploid. Data was further stratified based on established Society for Assisted Reproductive Technology (SART) age groups. Excluding mosaic results, 51.1%, 38.0%, 20.8%, 26.4%, and 10.5% were found in be euploid in patients under 35, 35-37, 38-40, 41-42, and over 42, respectively.
Limitations, reasons for caution: While the NICS platform has been previous validated, the proportion of mosaic calls can vary depending on thresholds established within the Yikon pipeline. Additionally, caution should be taken when generalizing results as certain clinical and embryologic practice parameters could affect DNA capture and successful amplification.
Wider implications of the findings: The NICS platform can be utilized successfully in a private setting. Embryos resulting as inconclusive initially can be successfully retested. Furthermore, the use of a non-invasive methods to identify embryo ploidy may reduce risk to the embryo, however this has yet to be determined.
Trial registration number: not applicable

P-513 A homozygous missense mutation in an highly conserved autosomal gene coding poly(A)-binding protein cause female infertility due to oocyte maturation arrest at GV stage

O. Okutman[1], A.S. Gürbüz[2], U. Büyük[3], B. Rinaldi[4], S. Friant[4], J. Muller[5], S. Viville[1]

[1]Hôpitaux Universitaires de Strasbourg- Nouvel hôpital civil, Laboratoire de diagnostic génétique- UF3472 Infertilité, Strasbourg, France ;
[2]Novafertil, IVF Center, Konya, Turkey ;
[3]Hibrigen Biyotecnology, Research and Development, Gebze-Kocaeli, Turkey ;
[4]Centre National de Recherche Scientifique CNRS- Université de Strasbourg, Department of Molecular and Cellular Genetics- UMR7156, Strasbourg, France ;
[5]Hôpitaux Universitaires de Strasbourg, Laboratoire de diagnostic génétique, Strasbourg, France

Study question: Is it possible to identify the new causative genetic mutation responsible of an oocyte maturation arrest in a Turkish consanguineous family via whole exome sequencing?
Summary answer: Whole exome sequencing reveals a homozygous missense mutation for the three affected sisters in a Turkish familiy with multiple loops of consanguinity.
What is known already: Women undergoing controlled ovarian hyper stimulation prior to in vitro fertilization (IVF) are treated by various protocols aimed at inducing multiple follicular growths. Approximately 20%–30% of oocytes collected for IVF are meiotically immature at the time of oocyte retrieval. The complete failure of all oocytes to mature *in vivo* is very rare, but known as causes of primary female infertility. Recently mutations in two genes, TUBB8 and PATL2 have been identified in women with oocyte maturation arrest. However, for approximately 60% of cases, underlying genetic factors are still largely unknown.
Study design, size, duration: We have recruited a Turkish familiy with multiple loops of consanguinity comprising 3 sisters with repeating oocyte maturation arrest at GV stage. Different stimulation protocols have been tried nevertheless collected oocytes failed to proceed into mature oocytes, even after extended in vitro culture. All three sisters have normal karyotype; however their brother was diagnosed with Down syndrome.
Participants/materials, setting, methods: Blood samples from all siblings, parents and available non-affected family members were collected after obtaining signed consent form. Genomic DNA was extracted from peripheral blood using Hibrigen DNA Extraction-Blood kit (Hibrigen, Istanbul, Turkey), according to

the manufacturer's instructions. Exome sequencing of three affected sisters was performed by Integragen Genomics via Illumina technology. Bioinformatics analysis was executed using IG Constitutionel DNA pipeline V4.0. Detected variants were also scored and ranked by VaRank tool.

Main results and the role of chance: For three samples, at least 4.8GB DNA sequence were generated with >99% of the target exome was represented with >25-fold coverage. Exome sequencing reveals a homozygous missense mutation in an autosomal gene (*STBG1*) for all three affected sisters. The mutation was confirmed and segregation has been shown in available family members via Sanger sequencing; parents, the cousin and one aunt are heterozygous whether second aunt is wild type for the substitution. The coding protein is well conserved during evolution from lamprey to human. The identified mutation is in RNA recognition domain, predicted as disease causing/deleterious through different online tools. KO female mice are infertile, and could not generate mature oocytes neither in vivo nor in vitro. Functional studies are ongoing; the effect of the mutation is studied on budding yeast.

Limitations, reasons for caution: Our study is limited to one family only and the phenotype is very rare. We still have to screen a cohort of patients presenting the same phenotype.

Wider implications of the findings: Our results provide a new understanding for the pathogenesis of oocyte maturation arrest and help us to identify reason for GV arrest in our patients. It may help to define novel therapeutic approach to patients with similar symptoms.

Trial registration number: Not applicable

P-514 From molecular identification to PGT-M of a novel primary ciliopathy due to biallelic mutations in the TOGARAM1 gene

D. Zuccarello[1], V. Morbidoni[2], E. Agolini[3], K.C. Slep[4], L. Pannone[5], E. Grosso[6], G. Gai[6], B. Dallapiccola[7], A. Novelli[3], S. Martinelli[8], E. Trevisson[2]

[1]Clinical Genetics Unit, Department of Women's and Children's Health- University of Padova, Padova, Italy ;
[2]Clinical Genetics Unit- Department of Women's and Children's Health- University of Padova, Istituto di Ricerca Pediatrica- IRP- Fondazione Città della Speranza, Padova, Italy ;
[3]Laboratory of Medical Genetics, IRCCS-Bambino Gesù Children's Hospital, Rome, Italy ;
[4]University of North Carolina, Department of Biology, Chapel Hill- NC, U.S.A. ;
[5]Department of Oncology and Molecular Medicine- Istituto Superiore di Sanità, Genetics and Rare Diseases Research Division- Ospedale Pediatrico Bambino Gesù, Rome, Italy ;
[6]Medical Genetics Unit, Città della Salute e della Scienza University Hospital, Turin, Italy ;
[7]Scientific Directorate, Bambino Gesù Children's Hospital and Research Institute, Rome, Italy ;
[8]Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

Study question: How to eliminate the risk of recurrence of a genetic condition with unknown molecular basis?

Summary answer: By identifying the causative gene, ascertained by robust functional studies, it is possible to offer PGT-M to the couple.

What is known already: Dysfunction in non-motile cilia is associated with a broad spectrum of developmental disorders characterized by clinical heterogeneity. Despite over one hundred genes have been associated with primary ciliopathies, with wide phenotypic overlap, some patients still lack a molecular diagnosis.

Study design, size, duration: Whole exome sequencing (WES) analysis, *in silico*, *in vitro* and *in vivo* functional studies.

Participants/materials, setting, methods: Unrelated young parents were referred following 2 TOP due to multiple fetal malformations, including microcephaly, severe cleft lip and palate, microphthalmia, and brain malformations. Array-CGH on fetal DNA was normal. WES analysis was performed on DNA from 2 fetuses and its parents, and *in silico*, *in vitro* and *in vivo* (in *C. elegans*) functional studies were carried out to explore the impact of finding mutations on protein structure and function.

Main results and the role of chance: WES analysis identified in both fetuses a compound heterozygous genotype in the orphan gene *TOGARAM1*: NM_015091.3:c.1102C>T [p.(Arg368Trp)], and c.3619C>T [p.(Arg1207*)], each inherited from one parent. *TOGARAM1* encodes a protein regulating microtubule dynamics, it is highly conserved throughout evolution and *che-12*, its orthologue in nematodes, is expressed in the cilium of a subset of sensory neurons.

Mutant worms recapitulating the patients' genotype showed a shorter cilium in sensory neurons and in vitro experiments confirmed aberrant tubulin binding, indicating a causative role of *TOGARAM1* variants in the pathogenesis of this novel primary ciliopathy, characterized by a spectrum of defects consistent with the Meckel-Gruber phenotype,including microphthalmia, hydrocephalus and cleft palate.

The molecular identification of this new genetic disorder allowed to offer the couple the opportunity to perform the preimplantation genetic diagnosis (PGT-M).

Limitations, reasons for caution: We cannot predict accurately the post-natal phenotype associated with biallelic *TOGARAM1* mutations, because we have only observed fetal cases. Further studies are warranted to better define the clinical picture of this new severe disorder.

Wider implications of the findings: Our data strongly support the role of *TOGARAM1* as a novel causative gene of primary ciliopathy, which thus should be included in diagnostic gene panels for this heterogeneous group of disorders.

Trial registration number: not applicable

P-515 PGT-A: Who and when? A systematic review and network meta-analysis of RCTs

A. Pantou[1], K. Sfakianoudis[2], E. Maziotis[3], P. Tsioulou[3], S. Grigoriadis[3], A. Rapani[3], P. Giannelou[2], M. Asimakopoulou[3], K. Nikolettos[4], T. Kalampokas[5], G. Kokkali[2], K. Pantos[2], N. Vlahos[6], M. Koutsilieris[3], M. Simopoulou[3]

[1]Genesis Genoma Lab, Molecular Genetics, Athens, Greece ;
[2]Genesis Athens Clinic, Assisted Conception Unit, Athens, Greece ;
[3]National and Kapodistrian Univerisity of Athens, Physiology, Athens, Greece ;
[4]Assisted Reproduction Unit of Thrace "Embryokosmogenesis", Assisted Conception Unit, Alexandroupoli, Greece ;
[5]Aberdeen Maternity Hospital- Fertility Center, Assisted Reproduction Unit, Aberdeen, United Kingdom ;
[6]Aretaieion University Hospital, 2nd Department of Obstetrics and Gynecology, Athens, Greece

Study question: What is the optimal practice for preimplantation genetic testing for aneuploidy (PGT-A) considering the patients' age and the best day to biopsy?

Summary answer: PGT-A employing complete chromosomal screening appears to be beneficial only when performed on the basis of trophoectoderm biopsy, addressing predominantly women over 35 years old

What is known already: Following literature search, two schools of thought-if not more-are emerging with regards to "when" and "how" PGT-A should be employed. A number of prospective and retrospective studies revealed controversial results concerning the effectiveness of PGT-A cycles during fresh or frozen ART cycles. On one hand, the advocators support that performing PGT-A holds remarkable promise with regards to successful embryo implantation, especially when focusing on the distinct group of women of advanced maternal age. On the other hand, no difference and even lower live birth rates have been demonstrated when opting for PGT-A in comparison to IVF cycles without PGT-A.

Study design, size, duration: A systematic review and meta-analysis of 10 published studies including women who underwent IVF-Embryo Transfer cycles following complete chromosomal screening (CCS) of the preimplantation embryo.

Participants/materials, setting, methods: A systematic search of the literature was performed in the databases of PubMed/Medline, Embase and Cochrane Central Library, limited to articles published in peer-reviewed journals up to June 2019. The initial search yielded 1819 studies. From the total yield, 215 studies were duplicates and 1501 were excluded on the grounds of not fulfilling criteria for inclusion. Following thorough full-text screening and citation mining a total of 10 studies were included in the current meta-analysis.

Main results and the role of chance: A total of 10 Randomized Control Trials employing CCS were identified. PGT-A improved ongoing pregnancy/live birth rates both in the pooled results (RR:1.29; 95%CI:1.07-1.56) and in the over

35 years old subgroup (RR:1.52; 95%CI:1.01-2.28), when compared to the control group of morphological assessment for embryo selection. Interestingly, no statistically significant difference was observed in the younger than 35 years old subgroup (RR:1.10; 95%CI:0.81-1.48), when compared to the control group. When comparing D3 and D5 biopsy no statistically significant difference was observed between the groups regarding ongoing pregnancy/live birth rates (RR:0.87; 95% CI: 0.59-1.29). When comparing D3 biopsy to the control group no statistically significant difference was observed between the groups regarding ongoing pregnancy/live birth rates (RR:1.10; 95% CI: 0.75-1.60). Interestingly, only D5 biopsy provided enhanced rates regarding ongoing pregnancy/live birth (RR: 1.26; 95% CI: 1.04-1.53) when compared to the control group.

Limitations, reasons for caution: The multifaceted nature of PGT-A success may stand as a valid limitation when unravelling results and drawing conclusions. When reporting on what truly makes a PGT-A cycle successful we should clearly distinguish between efficiency of diagnosis and successful outcome of the cycle leading to a healthy offspring.

Wider implications of the findings: The results of the present study indicate that PGT-A may improve clinical outcomes and live birth rates only when performed on women aged over 35 years old and on blastocyst stage embryos. However, further RCTs investigating the exact requirements to ascertain a beneficial effect of PGT-A should be conducted.

Trial registration number: Not applicable

P-516 Pregnancy after oocyte donation in a patient with NLRP7 mutation and a history of recurrent hydatidiform molar pregnancy

F. Scheffler[1], C. Cozette[1], J. Massardier[2], I. Touitou[3], J. Gondry[4], H. Copin[1], M. Ben Khalifa[1], R. Cabry-Goubet[1]

[1]CHU Amiens Picardie SUD, Service de médecine et biologie de la reproduction-cytogénétique et CECOS de Picardie, Amiens, France ;
[2]CHU Lyon Sud, Centre Français des Maladies Trophoblastiques- Hospices Civils de Lyon- Pierre Benite, Lyon, France ;
[3]CHU Montpellier, Cellules souches- plasticité cellulaire- médecine régénérative et immunothérapies- INSERM- Université de Montpellier- Département de génétique médicale- maladies rares et médecine personnalisée- CEREMAIA, Montpellier, France ;
[4]CHU Amiens Picardie SUD, Service de gynécologie et obstetrique, Amiens, France

Study question: Is oocyte donation the best option in case of genetic hydatidiform moles?

Summary answer: Oocyte donation appears to be the best option for a normal and successful pregnancy in women with a history of recurrent complete hydatidiform moles

What is known already: Molar pregnancies are benign trophoblastic diseases associated with a risk of malignant transformation. If aetiology remains mostly unknown, the risk of recurrent molar pregnancy is around 1.5% after one molar pregnancy and around 25% after 2 molar pregnancies. Genetic mutations have been described, increasing hugely this risk of trophoblastic diseases. In case of mutations, the probability to obtain a normal pregnancy is estimated around 1.8%. In patient with pathogenic mutations in the NLRP7 gene, only 3 normal pregnancies and deliveries after oocyte donation have been published.

Study design, size, duration: Case report

Participants/materials, setting, methods: We report the case of a Caucasian 30-year-old woman whose previous five spontaneous pregnancies had a negative outcome: a spontaneous miscarriage and 4 complete hydatidiform moles, without gestational trophoblastic neoplasia. There was no history of recurrent pregnancy failures in her family. Genetic testing revealed that the patient carried two pathogenic mutations in the *NLRP7* gene (Y318CfsX7 and c.2982-2A>G).

Main results and the role of chance: We proposed an oocyte donation in order to obtain a normal pregnancy. Four mature oocytes were done and two embryos transferred on day 3. This technic enabled a complication-free, singleton pregnancy that resulted in a healthy term live birth of a 2900g female, in spite of a pre-eclampsia at the end of the pregnancy. The pathological placenta examination was normal and we could observe a spontaneous normalization of BHCG. The couple is now waiting for another oocyte donation.

Limitations, reasons for caution: none

Wider implications of the findings: Women presented with mutations in the *NLRP7, KHDC3L or PADI6* genes are unlikely to obtain normal pregnancies, with a major risk of reproductive failure. Our case is the first one for our national trophoblastic disease center. It confirms the effectiveness of oocyte donation to prevent recurrent hydatidiform moles.

Trial registration number: not applicable

P-517 Analysis of segmental aneuploidy and mosaicism in the human blastocysts. Is there any difference in pregnancy rates?

J.A. Ortiz[1], B. Lledo[1], R. Morales[1], E. Garcia-Hernandez[1], J. Ten[2], J. Llacer[3], A. Bernabeu[3], R. Bernabeu[3]

[1]Instituto Bernabeu, Biología Molecular y Genética, Alicante, Spain ;
[2]Instituto Bernabeu, Embriología, Alicante, Spain ;
[3]Instituto Bernabeu, Medicina Reproductiva, Alicante, Spain

Study question: What are the characteristics, associated factors and the clinical pregnancy of the embryos with segmental alterations?

Summary answer: The ongoing pregnancy rates of mosaic segmental embryos are similar to those of euploid embryos, therefore, embryo transfer with segmental mosaicism could be considered.

What is known already: Chromosomal abnormalities in IVF embryos can be detected by PGT-A. By using NGS or aCGH, it is possible to detect segmental (subchromosomal) errors that occur when small portions of DNA are duplicated or removed from the embryo. The origin of the segmental alterations is mainly mitotic and occurs in the first embryonic divisions. Many of the characteristics of these alterations have not been studied, such as: proportion of chromosomes with segmental errors, hot spot regions, type of segmental errors, length of alterations, or percentage of aneuploid cells. Moreover, the effect of segmental mosaicism in the pregnancy rate is still controversial.

Study design, size, duration: A total of 5286 embryos from 1822 PGT-A cycles were included in this study (October 2014-April 2019). The trophoectoderm biopsies on D5/D6 blastocysts were analysed by aCGH (46%) or NGS (54%). The detection of segmental alterations was validated by the analysis of embryos from patients with structural chromosomal alterations. Embryos with ≤25% aneuploid cells were considered euploid, between 25-50% were classified as mosaic and aneuploid with >50%. Only euploid or mosaic embryos were transferred.

Participants/materials, setting, methods: The main indications for PGT-A were advanced maternal age, abnormal sperm FISH and recurrent miscarriage or implantation failure. Embryo analysis were performed using Agilent SurePrint G3-8x60K-CGH microarrays or Veriseq-NGS (Illumina), with previous whole genome amplification. The comparison of ongoing pregnancy rate between euploid embryos, segmental mosaic and full chromosome mosaic was performed by a binary logistic regression in which maternal and paternal ages, embryo quality and day of biopsy were introduced as confounding factors (SPSSv20.0).

Main results and the role of chance: A total of 5286 embryos were analysed (41.3% aneuploid and 2.6% not informative). Segmental alterations were detected in 12.4% of the analysed embryos, 42.9% of these embryos presented segmental deletions, 25.1% duplications and 32.0% both. The average size of the segmental imbalances was 56.87 Mb (8 to 170 Mb). Interestingly, sites of chromosome breakage associated with segmental errors were not random. A 58.2% of the segmental alterations were located near the telomeres, 7.4% in the proximity of the centromere, 4.7% in the middle of the arms and 29.7% affect a whole arm. Analysing the factors associated to segmental errors, we observed an inverse correlation among segmental alteration and maternal age: 15.3% in women until 35y versus 10.3% in >36y women ($p<0.0001$). No factors of paternal origin showed any association. On the other hand, embryonic quality showed a strong association with segmental errors (A: 10.3%; B14.5%; C:18.4%, D:18.2%;$p<0.0001$). We also analyzed the clinical data of embryos with segmental mosaicism versus embryos with full chromosome mosaicism or euploid in single embryo transfer cycles. The ongoing pregnancy rate was higher for segmental mosaics compared with full chromosome mosaics (42.1% vs 22.7%;$p=0.066$) and similar to cycles where euploid embryos were transferred (42.1% vs 33.9%;$p=0.254$).

Limitations, reasons for caution: The main limitation of this study is the use of different techniques for the PGT-A. The NGS is more sensitive and specific to detect segmental errors than aCGH. To avoid this bias, the analysis technique (aCGH or NGS) was introduced into the statistical analysis as a confounding factor.

Wider implications of the findings: Segmental-chromosomal alterations are relatively frequent in embryos and chromosome breaks close to the telomere are more likely. Maternal age and embryo quality are risk factors. Finally, ongoing pregnancy rates of mosaic segmental embryos are similar to euploid embryos, consequently the transfer of mosaic segmental embryos could be considered.
Trial registration number: Not applicable

P-518 Embryo morphology affects euploid blastocysts implantation

M. Gomez Peña[1], L. Kopcow[2], M. Rocha[2], G. Terrado[2], M. Papayannis[1], E. De Martino[1], M. Horton[2], F. Sobral[2], I. De Zúñiga[2], A. Oubiña[2], C. Bisioli[1]

[1]Pregna Medicina Reproductiva, ART Embryology, Buenos Aires, Argentina ; [2]Pregna Medicina Reproductiva, Clinical department, Buenos Aires, Argentina

Study question: Is blastocyst euploidy the only relevant factor to predict implantation?
Summary answer: Good quality euploid blastocysts have a better implantation and pregnancy potential.
What is known already: Euploidy has been regarded in the literature as the only guarantee of a successful implantation and live birth. Thus, irrespective of other embryological and clinical variables such as patient age, blastocyst morphology or biopsy day. This assertion, however, remains controversial.
Study design, size, duration: Retrospective comparative study of 222 elective single frozen-thawed blastocyst transfers after preimplantation genetic testing for aneuploidy (PGT-A) via next generation sequencing (NGS). Sampling occurred between June 2016 and September 2019.
Participants/materials, setting, methods: We compared implantation (IR), ongoing pregnancy (OPR) and miscarriage (MR) rates between:

- Embryo biopsy day 5 (n= 138) vs. day 6 (n= 84).
- Female age <37 yo (n= 89) vs ≥ 37 yo (n= 133)
- Excellent/Good (n= 138) vs. Fair/Poor (n= 84) quality embryos.

Student's t-test and Chi-squared test were used as appropriate.
Main results and the role of chance: IR (43.5% (n=64/147) vs 38.8 % (n=33/85); p=NS), OPR (39.2% (n=49/138) vs 29.7% (n=25/85); p=NS) and MR (5.8% (n=8/138) vs 7.2% (n=6/84); p=NS) were similar between embryos biopsied on D5 vs D6 respectively.

We also found no differences regarding patients age <37 vs ≥ 37 in IR (49.5% (n=45/91) vs 38.0% (52/137); p=NS), OPR (39.3% (n=7/89) vs 31.5% (n=7/133); p=NS) or MR (7.8% (n=35/89) vs 5.3% (n=42/133); p=NS). Excellent and good quality blastocysts achieved better IR (49.0 (n=70/143) vs 31.7% (n=27/85); p=0.01) and OPR (39.85% (n=55/138) vs 26.2% (n=22/84); p=0.02) than fair and poor ones. MR was not significantly different between different quality embryos (7.2% (n=10/138) vs 4.8% (n=4/84); p=NS).
Limitations, reasons for caution: This study has biases due to its retrospective design. The small sample size and the strong subjectivity among embryologists when grading blastocysts are additional limitations.
Wider implications of the findings: Although euploidy is considered as a main predictor of reproductive success our analysis shows that embryo grading might also play a role. Therefore diploid chromosome number alone might not be sufficient to predict a successful implantation.
Trial registration number: Not applicable

P-519 Freeze-all cycles with or without preimplantation genetic diagnosis for aneuploidy superior to fresh embryo transfer in normo-responders?

M. Karacan[1], M. Keskin[2], M. Kurek Eken[2], M. Yanık[1]

[1]Yeni Yuzyıl University Medical Faculty, Department of Reproductive Medicine and Infertility, Istanbul, Turkey ; [2]Yeni Yuzyıl University Medical Faculty, Department of Reproductive Medicine and Infertility, İstanbul, Turkey

Study question: Are freeze-all cycles with or without preimplantation genetic diagnosis for aneuploidy superior to fresh embryo transfer in normo-responders?
Summary answer: Live birth rate with single blastocyst transfer with or without PGT-A in freeze-all strategy is not superior to fresh ET in normoresponders.
What is known already: Cryopreservation of human embryos is now a routine procedure in assisted reproductive technologies for better embryo-endometrium synchrony and the lower risk of ovarian hyperstimulation syndrome at the same time resulted in live births increased. However, there are some concerns need to be evaluated in freeze-all strategy such as the efficacy in normal or poor responders. Aneuploidy is a leading cause of implantation failure and a significant cause of failure in IVF cycle, PGT-A has been deemed as a promising approach to improve pregnancy rate but on the other hand PGT-A requires consideration of multipl factors
Study design, size, duration: This retrospective study includes patients who underwent ICSI-ET procedures at Istanbul Yeni Yüzyil University Department of Reproductive Medicine&Infertility between January 2016 and December 2018. Fresh embryo transfers (n:72) frozen-thawed embryo transfer(FET) (n:264) and frozen-thawed embryo transfer after PGT-A (FET-PGT-A) (n:56) were analyzed.
Participants/materials, setting, methods: Women aged< 42 years with normal uterine cavity , normo-responders who had 5-14 oocytes at retrieval, single blastocyst transfer, ovulation induction with gonadotropin-releasing hormone antagonist (GnRH-a), sperm found in ejaculate, endometrial thickness ≥ 7 mm on the day of hCG injection in fresh ET cycles or of progesterone start in FET cycles ,first complete cycle in the center. Only blastocysts having normal karyotypes were transferred in PGT-A cycles.
Main results and the role of chance: A total of 392 couples who had fresh embryo transfer or a first frozen embryo transfer with or without PGT-A after freeze-all cycles were analyzed in the study. Fresh ET, FET and FET+PGT-A groups were comparable with regard to number of oocytes collected (8.8±2.3, 8.7±2.9 and 9.1±2.4) and fertilized (5.9±2.3, 6.1±2.2 and 6.3±2.1) as well as the number of blastocysts (2.3±1.2, 2.3±0.9 and 2.4±1.3) available for the transfer, consecutively.

Positivity of β-hCG level (>5 IU/L) was detected in 38 (52.7 %) women in fresh ET group, 168 (63.3 %) women in FET group and 33 (58.9 %) women in FET+PGT-A group following single blastocyst transfer (p>0.05).

All pregnancies were singletons and no severe OHSS was observed in all groups. Moderate OHSS was detected in 3 cases (4%) in fresh ET cycles and 11 cases (3.4%) in all FET cycles.

Live birth rates were 24 (33.3%) for fresh ET group, 98 (37.1%) for FET and 24 (42.8%) for FET+PGT-A group. Although it was higher in FET+PGT-A group compared to fresh ET group it did not reach statistical significance (p>0.05).
Limitations, reasons for caution: The limitations of our study include its retrospective nature, the lack of hormonal assessments in the follicular phase and imprecise allocation of patients into each group (physician's or patient's preference).Otherwise, it was an advantage that all procedures were performed by the same embryologist.
Wider implications of the findings: This was first study comparing fresh ET with FET with and without PGT-A in the literature.Although live birth rate was higher in PGT-A and FET group than fresh ET group but did not reach statistical significance. There is a need for further prospective randomised studies to confirm this subject properly.
Trial registration number: 2019/71

P-520 Developmental incompetent preimplantation embryo: a two Societies consensus

R. Ciriminna[1], A. Novelli[2], L. De Santis[3], M.G. Minasi[4], L. Rienzi[5], R. Gualtieri[6], D. Paoli[7], V. Pisaturo[8], C. Scarica[9], D. Zuccarello[10]

[1]AMBRA, On behalf of the Italian Society of Embryology- Reproduction and Research SIERR, Palermo, Italy ; [2]IRCCS-Bambino Gesù Children's Hospital Laboratory of Medical Genetics, On behalf of the Italian Society of Human Genetics SIGU, Rome-, Italy ; [3]San Raffaele Scientific Institute -Ob/Gyn IVF UNIT, On behalf of the Italian Society of Embryology- Reproduction and Research SIERR, Milan, Italy ; [4]Center for Reproductive Medicine- European Hospital, On behalf of the Italian Society of Embryology- Reproduction and Research SIERR, Rome, Italy ; [5]GENERA centres for Reproductive Medicine, On behalf of the Italian Society of Embryology- Reproduction and Research SIERR, Rome, Italy ; [6]University of Naples "Federico II"- Department of Biology, On behalf of the Italian Society of Embryology- Reproduction and Research SIERR, Naples, Italy ;

[7]Laboratory of Seminology - Sperm Bank "Loredana Gandini" - Sapienza" University of Rome, On behalf of the Italian Society of Embryology- Reproduction and Research SIERR, Rome, Italy ;
[8]International Evangelical Hospital- Reproductive Medicine, On behalf of the Italian Society of Embryology- Reproduction and Research SIERR, Genoa, Italy ;
[9]Casa di Cura Villa Salaria in partnership with Institut Marques- Center for Reproductive Medicine, On behalf of the Italian Society of Embryology- Reproduction and Research SIERR, Rome, Italy ;
[10]Clinical Genetics Units- University Hospital of Padova, On behalf of the Italian Society of Human Genetics SIGU, Padova, Italy

Study question: What is the definition of "developmental incompetent" preimplantation embryos, which can therefore be discarded, in the absence of a clear regulatory reference law?
Summary answer: SIERR and SIGU define as "developmental incompetent" a preimplantation embryo with specific characteristics related to ploidy, type of cell division, chromosomal and molecular status.
What is known already: In 2010 SIERR published the first document on the definition of "developmental incompetent embryos", in the absence of a specific indication in the Italian IVF Law 40/2004 on which embryo can be discarded at the end of IVF process.
Nine years later, SIERR, in collaboration with SIGU felt the need to update and implement the document, in the light of the development of new diagnostic capabilities related to modern technologies. The present consensus redefines the criteria according to which a preimplantation embryo can be defined as developmental incompetent.
Study design, size, duration: Five SIERR embryologists and 2 SIGU geneticists (a clinician and a biologist), representative of their respective scientific societies worked as a committee in 6 board meetings, through 2018 and 2019, in order to draw up the document. The consensus was then approved by the steering committee of the two scientific societies and made open through publication on their respective websites.
Participants/materials, setting, methods: Over a hundred between scientific articles and guidelines have been selected through systematic research of specific reference items. The most recent morphology and morphokinetics studies and the most advanced preimplantation genetic diagnosis technologies, allowed the working committee to develop standard criteria to define a developmental incompetent embryo**.**
Main results and the role of chance: The criteria to define a developmental incompetent embryo have thus been established as: a) presence of a number of pronuclei different from two; b) no further cell division 24 hours after the previous observation; c) blastocyst affected by complex aneuploidy; d) blastocyst affected by complete or partial monosomy in homogeneous form of an autosome; e) blastocyst affected by complete or partial trisomy in a homogeneous form of chromosomes: 1, 2, 3, 4, 5, 7, 10, 11, 12, 14, 19; f) blastocyst affected by polyploidy or haploidy; g) blastocyst affected by *in uterus* lethal monogenic disease.
Limitations, reasons for caution: Regarding the genetic criteria identified to define a developmental incompetent embryo, it is necessary to take into account the percentage of diagnostic error intrinsic to each technique in preimplantation genetic testing as well as the possible detection of chromosomal mosaicism, which varies according to the technique adopted.
Wider implications of the findings: This document fills a legislative gap of Italian law while providing a useful tool to support the choice to not transfer. It allows the IVF centers to discontinue these embryos from further culture, reducing the number of cryopreserved embryos, and opens to the donation of incompetent embryos to scientific research.
Trial registration number: not applicable

P-521 Cystic Fibrosis: Complete CFTR gene analysis vs. CFTR genotyping on sperm donors carrier screening

M. Molina Romero[1], A. Yoldi Chaure[1], P. Navas Bastida[2], M. Gañán Parra[3], Á. Vaquero Argüelles[1], J.L. Del Pico Sánchez[1], J.P. Ramírez López[1], J.A. Castilla Alcalá[1]

[1]CEIFER Biobanco, Genetic Department, Granada, Spain ;
[2]CEIFER Biobanco, Genetic Department, Córdoba, Spain ;
[3]CEIFER Biobanco, Genetic Department, Sevilla, Spain

Study question: The cystic fibrosis carrier screening on sperm donors, is better to do it through a complete analysis of the CFTR gene by NGS or by genotyping?
Summary answer: We recommend to study the CFTR gene by NGS for the cystic fibrosis carrier screening on sperm donors.
What is known already: In accordance with the legal regulations in force in Spain and under the European Union (EU) Directive on human tissues and cells, gamete donors should be screened (tested) for autosomal recessive genes known to be prevalent. Due to the high prevalence of cystic fibrosis, many scientific societies recommend the cystic fibrosis carrier screening for sperm donors. However, none has specified whether the CFTR gene should be studied by complete sequencing and non-directed analysis of variants or by using a panel of targeted variants (genotyping panel).
Study design, size, duration: It is a descriptive observational study, with 935 men aged 18-35 years, who were evaluated in the framework of a sperm donation programme in Spain, from January 2014 to June 2019.
Participants/materials, setting, methods: We did the complete study of the CFTR gen by Next Generation Sequencing (NGS) in 935 caucasian sperm donors in a Spanish private sperm bank. We have determined the frequency of sperm donors carrying pathogenic variants of the CFTR gene and we have analysed the detection rate of these carriers that would have been detected by applying the genotyping tests for CFTR gene mutations that are most commonly used in the assisted reproduction in Spain.
Main results and the role of chance: Of the 935 sperm donors, 159 (17%) were carriers of at least one pathogenic variant in the CFTR gene. The frequency, therefore, was approximately one in six. In total, 39 different variants were found. Of the 39 variants found in the sperm donors, 22 variants (56,41%) would not have been detected by any of the four genotyping tests. And only four of the 39 variants (10,27%) would have been detected by all of the genotyping tests.
Our study shows there is great heterogeneity in the pathogenic variants of the CFTR gene that are included in the genotyping tests most commonly used in the context of assisted reproduction. Less than 50% of the variants included in the panels are common to all these tests. The number of carrier donors detected by genotyping would be 8.2%-51,6% from total number of carrier donors detected by NGS.
Limitations, reasons for caution: The limitations of this study is that it is not a population study (men had to meet exclusion criteria with excellent semen parameters), and second that it is a relatively homogeneous population which may not extend to other ethnic groups. In consequence, our population is biased in this respect.
Wider implications of the findings: The NGS presents a higher detection rate of cystic fibrosis carrier than genotyping. There is a significant high reproductive risk when targeted panels are used.
We recommend to study the CFTR gene by NGS for the cystic fibrosis carrier screening on sperm donors to reduce the risk of having offspring affected.
Trial registration number: N/A

P-522 Effects of the Differences in Trophectoderm (TE) Biopsy Procedures on the Results of Next-Generation Sequencing (NGS) Analysis

K. Takeuchi[1], Y. Kuwatsuru[2], Y. Kuroki[2], Y. Fukumoto[2], M. Tokudome[2], H. Moewaki[2], E. Hashimoto[2], M. Watanabe[2], T. Iwakawa[1], T. Tabira[3], T. Kato[4], H. Kurahashi[4], M. Takeuchi[3]

[1]Takeuchi Ladies Clinic / Infertility Center, Infertility, Aira-shi- Kagoshima-, Japan ;
[2]Takeuchi Ladies Clinic / Infertility Center, laboratory, Aira-shi- Kagoshima-, Japan ;
[3]Takeuchi Ladies Clinic / Infertility Center, Gynecology, Aira-shi- Kagoshima-, Japan ;
[4]Fujita Health University, Division of Molecular Genetics. Institute for Comprehensive Medical Science, Toyoake-shi- Aichi, Japan

Study question: We examined how differences in TE biopsy procedures affect the incidence of chromosomal abnormalities, embryo transfer rate, and embryo mosaicism based on NGS analysis.
Summary answer: Of 204 embryos, 53 (25.9%) were appropriate for transfer, while 37 (18.1%) were completely normal. Embryo mosaicism was less frequent when more cells were biopsied.
What is known already: According to recent studies, results for NGS-based preimplantation genetic testing (PGT) and incidences of embryo mosaicism differ depending on facilities and procedures. Currently, we are performing embryo biopsies with a laser procedure, but this procedure has yielded a certain

percentage of mosaic embryos. Therefore, we considered it necessary to re-examine the effects of different TE biopsy procedures.
Study design, size, duration: The subjects comprised 18 patients (204 embryos) who underwent NGS-based PGT for structural rearrangements (PGT-SR) from 2016 to 2019. In addition, 24 surplus embryos were subjected to rebiopsy and diagnosed with NGS. The average age of patients at oocyte retrieval was 35.1 ± 2.5 years.
Participants/materials, setting, methods: Analysis results were examined retrospectively. In addition, we performed three patterns of TE biopsy: (A) excessive laser irradiation of cells, (B) no washing of the cells obtained by biopsy during tubing, and (C) the flicking method. These results were compared with those obtained previously.
Main results and the role of chance: The rate of obtaining a normal embryo was 18.1% (37/204), while that of obtaining embryos appropriate for transfer in accordance with ESHRE regulations was 25.9% (53/204). The incidence of a mosaic pattern was 26.4% (54/204). Besides structural abnormalities, many abnormalities were found in chromosomes 4, 15, 16, 21 and 22.

Analysis of the rebiopsied embryos showed that a mosaic pattern occurred in two samples with small numbers of collected cells in A, with no other effects observed. In B, a mosaic pattern occurred in two samples, with an overall increase of noise observed. In C, fewer mosaic patterns were observed in all embryos. However, the average number of cells collected with the flicking method and the laser procedure was 8.5 and 5.1, respectively, suggesting that the number of cells available for TE biopsy affected the occurrence of a mosaic pattern.
Limitations, reasons for caution: The sites of mosaic patterns may vary based on which site of the embryo is biopsied.
Little data is available because only PGT-SR has been approved in Japan.
Wider implications of the findings: The flicking method seemed to produce fewer mosaic patterns. More samples remained unaffected by excessive laser irradiation, suggesting cell numbers available for TE biopsy affected the occurrence of mosaic patterns. In our hospital, we perform TE biopsy under conditions where we can obtain more TE cells by extending culture periods.
Trial registration number: not applicable

P-523 Laser-assisted trophectoderm biopsy does not affect the embryo ploidy and reproductive outcomes in following single euploid embryo transfer

J.Y. Hsieh[1], Y.P. Chang[1], T.H. Chuang[1], M.J. Lee[1], H.L. Wang[1], H.H. Lai[1]

[1]Stork Ladies Clinic, Stork fertility center, Hsinchu, Taiwan R.O.C.

Study question: Does laser-assisted trophectoderm biopsy affect the embryo ploidy in preimplantation genetic testing for aneuploidy (PGT-A) cycles and reproductive outcomes in following single euploid embryo transfer?
Summary answer: Laser-assisted trophectoderm biopsy does not affect the embryo ploidy and following reproductive outcomes.
What is known already: The laser plays an important role in assisted reproductive technology, such as assisted hatching and embryo biopsy. High energy light is emitted by laser and the derived heat dissolves cells, thus making cell separation easier and convenient. Previous studies indicated that degradation of DNA occurs at high temperature, which makes the double strand break and disintegrate. Laser-assisted trophectoderm (TE) biopsy is still an invasive intervention to the embryos, whether the derived thermal effect impairs the embryo genetic materials and implantation capacity is not known already.
Study design, size, duration: The data was retrospectively collected from January to December in 2018, 1303 blastocysts were analyzed. The methods of TE biopsy were classified into laser-assisted (LA) and non laser-assisted (non LA) biopsy groups, and the ploidy states of embryos between the two groups were compared. Only patients with following single euploid embryo transfers were included in the analysis of reproductive outcomes.
Participants/materials, setting, methods: The output of laser was 400 mW within 0.2-0.24 msec. The biopsies were tested via Veriseq PGT-A (Illumina) and analyzed by BlueFuse Multi. The comparison of embryo ploidies between LA and non-LA groups was by age (<35, 35-37, 38-40, >40 years), and by embryo morphology: good (AA, AB, BA), median (BB), and fair (BC, AC). The definitions of euploid, mosaic, and aneuploid were based on aneuploid percentage: [euploid] <20%, [mosaic] 20-79%, and [aneuploid] >79%.
Main results and the role of chance: No significant difference was observed in ploidy distribution between LA and non-LA groups in overall patients (p=0.10). Furthermore, no significant difference was found in mosaic and aneuploid rates between the two biopsy groups in different age spans. Divided by embryo morphology, there were no significant differences in ploidy distribution between the two biopsy groups within the same morphologic grading: [good] mosaic rate (p=0.92) and aneuploid rate (p=0.94); [median] mosaic rate (p=0.57) and aneuploid rate (p=0.66); [fair] mosaic rate (p=0.68) and aneuploid rate (p=0.48). In the analysis of reproductive outcomes, biochemical pregnancy rate (BPR), clinical pregnancy rate (CPR), implantation rate (IR), and miscarriage rate (MR) between the two biopsy groups were also comparable: [BPR] 65.9% vs. 71.5% (p=0.37); [CPR] 56.0% vs. 59.2% (p=0.63); [IR] 53.8% vs. 56.2% (p=0.73); [MR] 2.2% vs. 3.8% (p=0.5).
Limitations, reasons for caution: The main limitation of this study is the retrospective nature. The challenges of PGT-A may occur during both the manipulation and ploidy testing, including biopsied cell number or amplification artifacts.
Wider implications of the findings: The study showed that utilization of laser for TE biopsy does not affect the embryo ploidy and reproductive outcomes, and routinely using laser-assisted biopsy would not increase the risk of embryo damage.
Trial registration number: not applicable

P-524 A randomized controlled trial comparing the two trophectoderm biopsy protocols for preimplantation genetic testing for aneuploidies (PGT-A): a prospective sibling oocyte study

M. Serdarogullari[1], O. Coban[1], F.K. Boynukalin[2], Z. Yarkiner[3], H. Bankeroglu[4], C. Simon[5], A. Capalbo[6]

[1]British Cyprus IVF Hospital, Embryology, Nicosia, Cyprus ;
[2]Bahceci Health Group, Obstetrics and Gynaecology, İstanbul, Turkey ;
[3]Cyprus Science University, Software engineering, Kyrenia, Cyprus ;
[4]British Cyprus IVF Hospital, Obstetrics and Gynaecology, Nicosia, Cyprus ;
[5]Igenomix Foundation, Medical, Valencia, Spain ;
[6]Igenomix Italia, Genetics, Marostica, Italy

Study question: Is there any difference between the two most frequently used biopsytechniques in PGT-A cycles?
Summary answer: Embryo quality was superior in the direct trophectoderm biopsy approach without cleavage stage zona opening while blastocyst biopsied rate was equivalent.
What is known already: Selecting euploid embryos for embryo transfer during assisted reproductive treatment, preimplantation genetic testing for aneuploidy has now been increasingly utilized worldwide. Nowadays with improving technology, trophectoderm biopsy (TB) application at blastocyst-stage and subsequent comprehensive genetic screening by next generation sequencing is well applied and adopted in PGT-A cycles in clinical practice. Based on zona pellucida handling where differ by the time of zona opening before biopsy, there are two approaches for TB. However, there is not enough comparative evidence in the literature indicating the superiority of one approach to another.
Study design, size, duration: This prospective sibling oocyte study was performed in British Cyprus IVF Hospital between May and December 2019. The inclusion criteria were: ≥ 6 fertilised zygotes using fresh donor or own oocytes, PGT-A cycles using NGS by TB. Primary outcome measure is the rate of biopsied blastocysts out of the number of utilized MII. Sample size was set at 1224 biopsied embryos to exclude a difference of 7% between the two groups (alfa 0.05, beta 0.80).
Participants/materials, setting, methods: This interim analysis includes 58 cycles and 686 embryos. For each cycle, normally fertilised oocytes were randomly assigned into two groups. Embryos in group-I had zona opening with laser on day 3 to make trophectoderm cells herniate through the hole before the biopsy while group-II contains embryos without zona opening. Main secondary outcome measures were blastocyst morphology at the time of biopsy, number of biopsied cells, time of biopsy procedure and genetic analysis outcomes.
Main results and the role of chance: In this study, 58 patients are taken into analysis consisting total of 686 embryos (355 embryos in Group I, 331 embryos in group II). Between the study groups, no statically significant difference in the number of 2PN (p= 0.852), blastocysts developmental rate (p=0.724), total number biopsied embryos (3.41±2.30 vs 2.91±2.19, p= 0.233), average biopsy time per embryo (p= 0.223) and the biopsied cell count (p= 0.151) were

observed. Regarding to the biopsied embryo quality on day 5 and 6, the proportions of embryos in three categories (best, good, poor) were found to be statistically significant different among study groups (p=0.044). In detail, the higher proportions of best quality embryos in group II (p=0.015) and the good quality embryos in group I (p=0.022) were observed while no statistically significant differences were found regarding poor quality embryos (p=0.565). Furthermore, between the groups the percentage of euploidy rate in analysed embryos did not show statically significant difference (51.6 vs 53.4 %, p= 0.758). In detail, there was not significant discrepancy in no results rate (1.3 vs 1.7 %, p=0.750), type of aneuploidies as mosaicism (6.5 vs 9.3 %, p= 0.557) and complex aneuploidies (24.7 vs 20.4 %, p= 0.564) among groups

Limitations, reasons for caution: This is an interim and our results should be taken as preliminary; therefore, the clinical significance of the finding needs to be validated in the planned sample size. However, the study is already sufficiently powered to exclude major differences in the primary outcome measure.

Wider implications of the findings: This is the first study to evaluate different biopsy approaches by using sibling oocyte design that could assist embryologist while seeking more convenient approach during embryo biopsy for PGS if adequate evidence reached.

Trial registration number: non applicable

P-525 Do maternal and embryo MTHFR gene polymorphisms have any influence on embryo chromosomal abnormalities and the ongoing pregnancy rate?

G. Eva M.[1], R. Morales[1], H. Codina[1], A. Cascales[1], B. Lledo[1], J.A. Ortiz[1], J. Ten[2], J. Llacer[3], A. Bernabeu[3], R. Bernabeu[3]

[1]Instituto Bernabeu, Molecular Biology and Genetics, Alicante, Spain ;
[2]Instituto Bernabeu, Embriology laboratory, Alicante, Spain ;
[3]Instituto Bernabeu, Reproductive Medicine, Alicante, Spain

Study question: Is there any relationship between maternal and embryo *MTHFR* polymorphisms (c.677C>T and c.1298A>C) and embryo chromosomal abnormalities or the ongoing pregnancy rate of euploid embryos?

Summary answer: Maternal and embryo *MTHFR* polymorphisms do not increase the aneuploidy rate, as well as they are not correlated with the ongoing pregnancy rate.

What is known already: MTHFR enzyme is involved in folic acid metabolism and play a role in reproduction. Many investigations have linked point mutations in the *MTHFR* gene with embryo alterations and problems in pregnancy. The single-nucleotide-polymorphisms 677C>T and 1298A>C are the most relevant. Maternal genotype has been associated with embryo aneuploidy rate in previous studies. Moreover, a negative correlation between the embryo genotype and the implantation rate has been described. However, it is still a controversial issue. The aim of our study was to investigate the effect on embryo aneuploidies and mosaicism and the correlation with pregnancy rate.

Study design, size, duration: *MTHFR* genotype (677C>T and 1298A>C) was analysed in 77 women who performed an IVF/ICSI cycle with their own oocytes and also carried out a preimplantation genetic testing for aneuploidy (PGT-A) from January 2016 until December 2018. Moreover, *MTHFR* genotype was analysed in the 189 embryos biopsied in the PGT-A cycles from these patients. In addition, 218 DNA samples from trophoectoderm biopsies belonging to a different group of patients were also genotyped for *MTHFR* polymorphisms.

Participants/materials, setting, methods: *MTHFR* genotyping was determinated with TRF-plus Thrombosis Risk Panel (Elucigene Diagnostics). Embryo chromosomal analysis was performed using Agilent SurePrint G3-8x60K-CGH microarrays or Veriseq-NGS (Illumina), with previous whole genome amplification. The main parameters analysed among *MTHFR* genotypes (both in embryos and in patients were the embryo aneuploidy and mosaicism rate. Additionally, the ongoing pregnancy rate of euploid blastocysts (n=243) was assessed. The differences between groups were evaluated by chi-square and binary logistic regression and (SPSSv20.0).

Main results and the role of chance: The aneuploidy rates obtained were comparable in embryos coming from patients with homozygous normal genotype and those embryos from women with at least one mutated allele (54.7% in 677CC vs. 30.2% in 677CT/TT, p=0.058 and 37.8% in 1298AA vs. 42.7% in 1298AC/CC, p=0.212). Furthermore, no differences were observed in mosaicism rate (24.0% in 677CC vs. 13.8% in 677CT/TT, p=0.345 and 17.1% in 1298AA vs. 17.3% in 1298AC/CC, p=0.865).

If we consider the embryo *MTHFR* genotype, the aneuploidy rate was 22.3% in 677CC blastocysts vs. 15.7% in blastocysts with at least one mutated allele (p=0.464), and 17.9% in embryos 1298AA vs. 18.3% in embryos 1298AC/CC (p=0.988). On the other hand, the incidence of embryo mosaicism was 13.5% in 677CC group vs. 5.4% in 677CT/TT group (p=0.019) and 7.5% in 1298AA vs. 9.1% in 1298AC/CC group (p=0.817). The only significant difference was observed in mosaicism rate among 677C>T genotype.

The ongoing pregnancy rate was compared between different embryo genotypes and no differences were found (41.9% in 677CC vs. 39.5 in 677CT/TT, p= 0.611 and 37.4% in 1298AA vs. 43.6% in 1298AC/CC, p= 0.276). Morevoer, no differences were observed among different maternal genotypes.

Limitations, reasons for caution: The limited sample analysed precludes definite conclusions. Larger sample size studies are warranted to confirm our findings. Future studies focused on genes involved in embryo aneuploidy, implantation and pregnancy development are needed.

Wider implications of the findings: Our analysis suggests that neither maternal nor embryo MTHFR genotype influences the aneuploidy or ongoing pregnancy rate. The only correlation was observed in mosaicism rate among 677C>T embryo genotypes. Further research is need to clarify the role of this genotype on the processes that lead to mosaicism in the embryo.

Trial registration number: not applicable

P-526 Consistent results of non-invasive pre-implantation genetic testing for aneuploidy (niPGT-A) of human embryos using two different techniques for chromosomal analysis

B. Lledo[1], R. Morales[1], J.A. Ortiz[1], A. Rodriguez-Arnedo[2], J. Ten[2], J.C. Castillo[3], A. Bernabeu[3], J. Llacer[3], R. Bernabeu[3]

[1]Instituto Bernabeu, Molecular Biology, Alicante, Spain ;
[2]Instituto Bernabeu, Embriology, Alicante, Spain ;
[3]Instituto Bernabeu, Reproductive Medicine, Alicante, Spain

Study question: Are the results of niPGT-A similar when analysed with different genetic techniques?

Summary answer: Consistent results (diagnosis and concordance rate) are obtained with different genetic techniques used for niPGT-A, thus suggesting discordances might be attributable to mosaicism or DNA-contamination.

What is known already: The concept of niPGT-A has generated huge interest in reproductive medicine. Variable success and concordance rates have been reported and discordances seem related to embryonic mosaicism, favoured elimination of aneuploid cells, DNA contamination and the method used for analysis (amplification/detection). The niPGT-A efficacy has been restricted by technical complications associated with the low quantity and quality of the DNA, presenting technical challenges for genetic analysis. It is not clear which method is the most appropriate. The aim of this study was to assess the accuracy of niPGT-A by comparing two different chromosomal analysis techniques.

Study design, size, duration: A prospective blinded validation study was performed from September 2018 to December 2019, including 302 chromosomal analyses. Two hundred and seventy-six corresponded to trophectoderm biopsies (TE) from couples who attended our clinic for PGT-A and whose correspondent spent blastocyst medium (SBM) was evaluated by two methods. Eight blank-medium were evaluated for DNA contamination detection. Finally, to investigate the diagnostic discrepancies: TE-aneuploid embryos were thawed and re-analyzed using TE and inner cell mass (ICM) biopsies.

Participants/materials, setting, methods: Embryos were cultured in continuous media (Global Total LP) until D3, washed three times and cultured again until embryo biopsy. Embryos were biopsied on D5 or D6. TE analysis was carried out using Veriseq (Illumina®). Frozen euploid embryos according to the TE biopsy were thawed and transferred in the subsequent cycle. SBM collection was done following TE biopsy and samples were stored at -80°C. SBM chromosomal analysis was performed using Veriseq (Illumina®) and NICS (Yikon®).

Main results and the role of chance: We obtained genetic results in 96.8% of TE-samples vs 90.4% in SBM using both techniques(p>0.05). The mosaicism rate was higher in SBM (30.4% SBM-Yikon;28.3% SBM-Veriseq) compared to TE-biopsies (14.1%) (p= p=0.013; p=0.031) regardless of the technique used. We performed comparisons between both SBM techniques showing 95.2% of consistency in the diagnosis. Regarding diagnostic concordance (euploid-euploid vs aneuploid-aneuploid) between each SBM-technique and TE-biopsy, we obtained 74.6% SBM-Yikon vs 72.3% SBM-Veriseq. However, when we considered embryos

biopsied on D6 these rates reached up the 92.0% and 86.5%, respectively. Analysing the full chromosome concordance, the cytogenetic results were exactly the same as the TE-biopsy in 45.2% SBM-Yikon and 41.7% SBM-Veriseq. Moreover, in 20.1% SBM-Yikon and 23.3% SBM-Veriseq the results were discordant only in mosaicism diagnosis. The remaining were partial (22.6% SBM-Yikon vs 23.3% SBM-Veriseq) and complementary (4.8% SBM-Yikon vs 3.3% SBM-Veriseq) discordances. To identify the cause of discrepancies we reanalysed TE-aneuploid embryos, 55.6% of the discrepancies were due to DNA contamination (maternal origin), 22.2% to embryo mosaicism, 11.1% to low resolution in SBM-Yikon and 11.1% low resolution in both techniques. Finally, we analysed the IVF outcome, the ongoing pregnancy rate was 50% for TE-euploid-SBM-Yikon-aneuploid vs 33.3% TE-euploid-SBM-Veriseq-aneuploid.

Limitations, reasons for caution: The main limitation of the study is the sample size, especially for the subgroup analysis. Larger, prospective studies are warranted to draw definite conclusions on the accuracy of niPGT-A in representing the genetic constitution of the whole embryo with potential to improve pregnancy outcomes.

Wider implications of the findings: Diagnostic concordance between PGT-A and niPGT-A seems independent of the technique used for genetic analysis. Interestingly, concordance was higher for day-six-biopsied embryos. Therefore, niPGTA may be influenced by factors such as DNA contamination and embryo mosaicism. Optimization of culture conditions and medium retrieval constitutes potential targets to improve NIPGT-A reliability.

Trial registration number: NCT03879265

P-527 Morphine treatment prevents the X chromosome inactivation and maintains a long-term repression by SMCHD1 epigenetic regulator.

N. Subiran[1], I. Muñoa-Hoyos[1], M. Araola-Lasa[1], I. Urizar-Arenaza[1], M. Gianzo[1], J. Irazusta[1]

[1]University of Basque Country, Physiology, Bilbao, Spain

Study question: To elucidate how morphine can cause stable epigenetic changes on X-chromosome inactivation (XCI) process that can be memorized and persist in the long term.

Summary answer: Chronic morphine treatment prevents the XCI through PRC2 and *Dnmt1* in the short term and Smchd1 maintains this long-term repression in mESC and blastocysts.

What is known already: XCI is an important epigenetic process that exemplifies the developmentally controlled formation of silent chromatin, where one of the X chromosomes in females is inactivated for dosage compensation. The XCI involves several steps, which implies the initiation of chromosome-wide silencing by XIST, the formation of a repressive compartment through Polycomb complexes and finally the maintenance of the stable repression by DNA methylation and other epigenetic regulators in which SMCHD1 plays an important role. Although XCI is part of normal development, there is a big concern in understanding how environmental factors can cause stable epigenetic changes that lead to health problems.

Study design, size, duration: To evaluate if morphine can induce cellular epigenetic memory, OCT4-reported mESCs were chronically treated with morphine during 24h, 10^{-5}µM (P1). After morphine removal, mESCs were seeded and maintained in culture for 48h (P2) and 96h (P3). To elucidate the role of morphine in early embryo development, two cell- embryos stage were chronically treated with morphine for 24h and *in-vitro* cultured up to the blastocyst stage in the absence of morphine.

Participants/materials, setting, methods: Transcriptomic analyses and H3K27me3 genome wide distribution were carried out by RNA-Sequencing and Chip-Sequencing respectively. Validations were performed by RNA-RT-qPCR and Chip-RT-qPCR.

Main results and the role of chance: Dynamic transcriptional analyses identified a total of 932 differentially expressed genes (DEGs) at P1 and, in the absence of morphine, 1196 DEGs at P2 and 2138 DEGs at P3, providing strong evidence of the existence of transcriptional cellular memory induced by morphine. Chronic morphine led to a down-regulation of epigenetic regulators important for XCI, such as PCR2 complex and *Dnmt1* in the short term. High-throughput screening approaches showed up *Smchd1* as a key regulator of a long-term epigenetic cellular memory induced by morphine. Morphine caused a down-regulation of *Smchd1* gene expression that can persist over the time after morphine withdrawal. ChIP-sequencing analysis confirms a decrease of H3K27me3 enrichment on the whole coverage of the X chromosome. The *Smchd1* gene expression was also down-regulated in *in-vitro*-morphine-treated blastocysts, preventing the XCI in early embryo. This is consistent with the increase in blastocyst rate observed after morphine chronic treatment. Our results clearly prove that chronic morphine treatment prevents the XCI, modifying PRC2 and *Dnmt1* gene expression in the short term and maintaining the repression upon *Smchd1* in the long term. SMCHD1 plays a role as a key regulator of epigenetic cellular memory induced by morphine, conferring to the cell a stable activate state

Limitations, reasons for caution: To perform the *in-vitro* analysis.

Wider implications of the findings: Morphine alters normal X chromosome-wide silencing, providing insights into the epigenetic mechanisms underlying cell memory induced by morphine and establishing the bases to understand how environmental factors can cause epigenetic changes, which leads to health problems or diseases.

Trial registration number: DTS18/00142

P-528 Cigarette smoke alters the expression of genes involved in the inflammatory response in mouse uterus

M.C. Budani[1], E. Carletti[2], G.M. Tiboni[2]

[1]University "G. d'Annunzio" Chieti-Pescara- Italy, Department of Medicine and Aging Sciences, Chieti, Italy ;
[2]University 'G. d'Annunzio' Chieti-Pescara- Italy, Department of Medical- Oral and Biotechnological Sciences, Chieti, Italy

Study question: What are the effects of a whole body exposure to cigarette smoke on stress and toxicity pathways in the mouse uterus?

Summary answer: The *in vivo* cigarette smoke exposure induced an over-expression of pro-inflammatory cytokines and a down-regulation of extracellular matrix metallopeptidases in mouse uterus.

What is known already: Cigarette smoking was associated to a lower chance of clinical pregnancy and live birth rates, and a dramatic increase in the incidence of spontaneous miscarriages in women. The noxious effects of cigarette smoke exposure have been studied especially in the ovaries. Research linking smoking to uterus damage mainly relies on studies *in vitro* testing a single cigarette smoke-derived component. Smoke components seemed to exert adverse effects on endometrial angiogenesis in human primary endometrial cells and in mouse endometrial cells as well and on uterine cells proliferation with a role in decreasing the endometrial thickness.

Study design, size, duration: Female C57BL/6 mice (aged 8 weeks) were *in vivo* exposed to the smoke originated by 3 cigarettes once a day, 7 days/week for 2 months using a specific whole-body mainstream exposure system. Control mice were placed in a restrainer and exposed to room air only for the same time period.

Participants/materials, setting, methods: At the end of exposure, mice were euthanized and the uteri collected. From these, the extracted RNA was used to perform a real-time PCR using an array consisting of a panel of 84 key genes involved in stress and toxicity pathways. Representative genes with >2 fold differential expression identified in the array were validated by real-time PCR gene expression assays.

Main results and the role of chance: No adverse changes in body conditions and atypical behaviors were noted as result of cigarette smoke exposure. The genes that were significantly up-regulated in the gene array were as follows: reactive protein C (Crp, p-value < 0.05), growth arrest and DNA-damage-inducible45γ (Gadd45γ, p-value < 0.05), interferon γ (Ifnγ, p-value < 0.01), and interleukin1α (Il1α, p-value < 0.005). Cigarette smoke was associated in the gene array with a down regulation of matrix metallopeptidase-9 (Mmp9, p-value < 0.05) compared to control group. The qRT-PCR analysis validated the differential expression profile of the representative genes deregulated at least two folds in the array.

Limitations, reasons for caution: The experimental nature of the study limits the capacity to extrapolate to the human field.

Wider implications of the findings: This is the first study showing the capacity of *in vivo* cigarette smoke exposure in inducing an over-expression of inflammatory genes and concomitant down-expression of MMP9 in mouse uterus. These data support the idea that inflammation cigarette smoke-derived may interfere with the tissue remodeling process responsible for the embryo implantation.

Trial registration number: not applicable

P-529 To flick or to pull? The effect of trophectoderm biopsy methodology on mosaicism incidence

L. Coll[1], M. Parriego[1], S. Mateo[1], B. Carrasco[1], S. García[1], M. Boada[1], B. Coroleu[1], A. Veiga[1]

[1]Dexeus Mujer, Obstetrics- Gynecology- and Reproductive Medicine, Barcelona, Spain

Study question: Is mosaicism incidence related to differences in biopsy methodology?
Summary answer: Mosaicism incidence is not related neither to the number of laser pulses during biopsy nor to the use of flicking/pulling methodology.
What is known already: Chromosomal mosaicism may be an inherent feature in preimplantation human embryos. However, reported incidences vary widely among different IVF and PGT-A settings. Although such differences could be attributed to the use of different diagnostic methodologies and criteria, settings with the same diagnostic platforms have observed different mosaicism incidence. This points to the fact that extrinsic factors can also have an impact. Moreover, it has been speculated that the biopsy procedure itself and biopsied sample handling could induce the generation of artefactual mosaicism leading to an overestimation of such phenomenon.
Study design, size, duration: This is a prospective observational study analysing the chromosomal constitution of 484 trophectoderm biopsies from PGT-A cycles performed from May 2019 to January 2020 in a single IVF setting. Mosaicism incidence was evaluated in relation to different biopsy methodologies.
Participants/materials, setting, methods: Trophectoderm biopsies were performed by flicking or pulling depending on embryo characteristics and analyzed by NGS. Data on the quality of the biopsied sample (with or without lysed cells) was analysed with regards to biopsy methodology. Additionally, the number of laser pulses and the biopsy methodology (flicking or pulling) were analyzed for mosaicism incidence. Time from freezing to amplification was also assessed in relation to mosaicism.
Main results and the role of chance: In 73.8% of cases (357/484), ≤3 laser pulses were needed to obtain a biopsy. Moreover, in 34.3% of biopsies (166/484), the trophectoderm sample was obtained by pulling methodology vs 65.7% (318/484) in which the methodology was flicking. The percentage of samples showing signs of lysis was higher when flicking methodology was used compared to pulling (20.8% vs 10.8%; p-value = 0.01).
The incidence of mosaicism did not vary among different biopsy operators (p=0.1). In relation to biopsy methodology, similar mosaicism incidence was observed for pulling and flicking methods (13.9% vs 16.0%; p-value =0.5). The number of laser pulses applied was not related to mosaicism either (3.30±0.75 in mosaic embryos vs 3.33±0.66 in non-mosaic embryos; p-value = 0.67).
Concerning biopsied sample storage, no differences were observed regarding the elapsed time from biopsy to amplification (10.4d ±10.4 in mosaic embryos vs 11.0 d ±13.8; p-value=0.7).
Limitations, reasons for caution: These are preliminary results. For a deeper analysis controlling for potential confounding factors more data are needed. Embryologists were homogeneously trained and followed the same SOPs. As a consequence, there was small variability in biopsy methodology.
Wider implications of the findings: Each PGT setting can choose its own procedures as long as trophectoderm biopsy is performed within homogeneous methodology and high quality standards. However, it remains to be assessed whether different biopsy methodologies have an effect on embryo reproductive outcome.
Trial registration number: Not applicable

P-530 Euploidy and embryo quality with single step medium with or without renewal post assisted hatching (AH) in a PGT-A program using Embryoscope dishes.

A. Delgado Mendive[1], A. Mercader[1], L. Escrich[1], D. Beltran[1], A. Tejera[1], N. Grau[1], F. Insua[1], M. Nohales[1], A. Mifsud[1]

[1]Instituto Universitario IVI Valencia, ivf and pgd laboratory, Valencia, Spain

Study question: Is the culture medium affected by the heating of the EmbryoscopeÒ dish after the AH and how could it influence embryo development and euploidy rates?
Summary answer: The results regarding euploidy and embryo quality between both groups were similar and AH did not seem to modify the conditions of the culture medium.
What is known already: According to the embryonic culture protocol, the culture medium is usually renewed on day 3 of development, although currently the use of a single step medium makes it unnecessary. In the past, different techniques were used to perform AH. Nowadays in most laboratories, the use of laser has been unified. There have been studies on the technique and its usefulness, but due to its heterogeneity, there have been no conclusive results. In this way there are no specific data related to the heating of the dishes and the culture medium and their effect on the results.
Study design, size, duration: Three months retrospective study (October 2017- December 2017) with 190 patients of the PGT-A program. They were divided in two post-hatching categories on day 3: AHR (assisted hatching renewal) and AHNR (assisted hatching no renewal).
Participants/materials, setting, methods: The AHNR group included 94 patients and AHR group included 96 patients. The AH was performed by laser Octax Ò on day 3 directly on the EmbryoscopeÒ dish and the biopsy was done at blastocyst stage. Chromosomal analysis method was NGS (Thermo Scientific, INC., USA). For embryonic categorization, the ASEBIR classification was used being A cathegory the best prognosis blastocyst and D the worst. The statistical analysis was performed by Fisher's exact test.
Main results and the role of chance: A total of 1505 embryos were obtained, of which 238 were biopsied in the AHNR group and 242 in the AHR group. For the AHNR group, 105 blastocysts were euploid (44.12%) and 133 aneuploid (55.88%). Regarding the 242 of the AHR group, 110 were euploid (45.45%) and 132 aneuploid (54.55%). With reference to the the morphology by categories, the data for AHNR were A: 5.35%; B: 48.18%; C: 27.26%; D: 19.21% and for AHR A: 5.97%; B: 49.61%; C: 26.25% and D: 18.17%. No statistical differences were found between both groups (p>0.05).

Thus, it seems that renewing the culture medium on day three of development using single step medium does not provide an improvement with respect to the morphology of the blastocysts, as well as regarding the percentage of euploidy. Therefore, we can conclude that the single step medium is a reliable option for the PGT-A program since the AH does not modify the culture conditions.
Limitations, reasons for caution: Retrospective study and the limited number of cases. Further studies are necessary to corroborate these results. In addition, study conditions may vary from other laboratories, for example: culture conditions, the power of the laser and the medium used for embryo culture. Therefore, results may not be comparable between laboratories.
Wider implications of the findings: The introduction in the laboratories of the time-lapse technology with new and modern dishes as well as the use of one step media, has meant a change in the working protocols. The combination of both factors with AH requires more studies that show the possible influence on clinical outcomes.
Trial registration number: 1503-VLC-017-AM

P-531 Differential endometrium DNA methylation and gene expression patterns underline mechanisms of reproductive failure in women with unexplained recurrent miscarriage (RM) and recurrent implantation failure (RIF)

R. Zhang[1], S.W. Hung[1], X. Chen[2], T.C. Li[2], C.C. Wang[1]

[1]Chinese University of Hong Kong, Obstetrics and Gynecology, Hong Kong, Hong Kong ;
[2]Prince of Wales Hospital- The Chinese University of Hong Kong, ART unit-Obstetrics and Gynecology, Hong Kong, Hong Kong

Study question: What is the underlying mechanism cause the differential gene expression in endometrium in women with unexplained RM and RIF, and causes their endometrial receptivity disorder?
Summary answer: Differential endometrium DNA methylation-regulated genes for reproductive failure were identified. RIF and RM were associated with abnormalities in endometrium cell adhesion and metabolic pathway, respectively.
What is known already: Unexplained RM and RIF are two major challenging reproductive failure attribute to endometrium. Those women have normal uterus, parental karyotype and basal hormone level; negative for autoimmune antibodies and thromobophilia, uNK and plasma cells, and even good embryo quality. Abnormal endometrium in molecular levels may contribute to the failure. Our previous study of endometrium RNA sequencing (RNAseq) identified differential transcriptome profiles between RM and RIF. Epigenetics can modify gene expression without change in gene structure. Evidences have shown that

abnormal DNA methylation modification will result in abnormal endometrial genes expression and thereby reproductive failure.
Study design, size, duration: This is a prospective study of DNA methylation and gene expression of endometrium in genomic scale. Fertile control women and unexplained RM and RIF undergoing IVF treatment were recruited. 5 endometrium samples in each group were included for gene discovery study. Another 10 endometrial samples in each group were included for validation and functional studies. All the samples were collected and analysed in 2019.
Participants/materials, setting, methods: Endometrial samples were obtained from control fertile women, and women with RM and RIF. All samples were collected on LH+7 in natural cycle and stored in -80°C before assays. Whole genome bisulfite sequencing (WGBS) was used to assess DNA methylation and RNA sequencing (RNAseq) was used to assess transcriptome pattern in the same endometrium samples. Differential methylated genes (DMG) were selected and target methylation specific-PCR (MS-PCR), RT-qPCR and immunostaining were used to validate the results.
Main results and the role of chance: RNAseq identified 969 differentially expressed genes (DEGs) between RIF and controls, 370 DEGs between RM and controls, and 872 DEGs between RM and RIF. WGBS detected higher overall methylation level in RIF and controls than RM. 157989 differentially methylated regions (DMRs) were found in RM versus controls, 85834DMRs in RIF versus controls, and 133116 DMRs in RIF versus RM in all genome regions. If promoter region only, we found 79 differentially methylated genes (DMGs) in RIF versus controls, and 51 DMGs in RM versus controls, and 76 DMGs in RM versus RIF. For RIF, most DMGs were enriched in calcium ion binding and plasma membrane mainly into cell adhesion pathway. For RM, most DMGs were enriched in protein binding, integral component of membrane and mitotic cell cycle mainly into metabolic pathway. Based on biological and molecular functions, 4 DMGs including AQP3, AURKB, CRISP2, CRYBB2 were chosen for validation and functional studies.
Limitations, reasons for caution: Although we attempted to eliminate embryo factors by embryo morphology grading before transfer, molecular composition of the embryos was still unknown. Apart from DNA methylation, other epigenetic mechanisms, such as histone acetylation and methylation; and non-coding RNA, on the endometrium abnormalities are still not known.
Wider implications of the findings: Important endometrium genes regulated by DNA methylation for the reproductive failure were identified. This not only increases the understanding one of the underlying molecular mechanism for the conditions, but also provides potential therapeutic targets to improve the pregnancy outcomes in these women.
Trial registration number: CREC 2016.160-T

P-532 Identification of a unique epigenetic profile in women with diminished ovarian reserve

K.W. Olsen[1], J. Castillo-Fernandez[2], A.C.H. Chan[3], N.L.C. Freiesleben[4], A. Zedeler[5], M. Bungum[6], A. Cardona[7], J. Perry[7], S. Skouby[1], R. Borup[3], E.R. Hoffmann[3], K. Gavin[2], M.L. Grøndahl[1]

[1]Copenhagen University Hospital Herlev, Department of Obstetrics and Gynaecology- Fertility Clinic, Herlev, Denmark ;
[2]Babraham Institute, Epigenetics Programme, Cambridge, United Kingdom ;
[3]Faculty of Health and Medical Sciences- University of Copenhagen, DNRF Center for Chromosome Stability- Department of Cellular and Molecular Medicine, Copenhagen, Denmark ;
[4]VivaNeo Fertility Clinics, Stork IVF Clinic A/S, Copenhagen, Denmark ;
[5]Copenhagen University Hospital Hvidovre, Department of Obstetrics and Gynaecology- The Fertility Clinic, Hvidovre, Denmark ;
[6]Skåne University Hospital, Reproductive Medicine Centre, Malmö, Sweden ;
[7]University of Cambridge Addenbrooke's Hospital Cambridge, Medical Research Council Epidemiology Unit, Cambridge, United Kingdom

Study question: Does the epigenetic profile of mural granulosa cells (MGC) and leukocytes from women with diminished ovarian reserve (DOR) differ from other women?
Summary answer: MGC from human ovarian follicles have a distinctive epigenetic profile in women with DOR. The same does not apply for leukocytes.
What is known already: DOR is defined as a reduced number of oocytes and can be *physiological* due to advanced reproductive age or *pathological* due to premature loss of fertility. Previous studies have found an increase in apoptosis of MGC from women with DOR (Fan et al. 2019) and we have previously shown thatMGC have a distinctive epigenetic ageing profile compared with other somatic cell types (Olsen et al. under review). But whether the MGC from women with DOR differ from MGC from other women is not known.
Study design, size, duration: Multicenter cohort study based on a retrospective analysis of prospectively collected data (September 2016 – June 2018) and material (blood and MGC) derived from 119 healthy women undergoing IVF or ICSI treatment following controlled ovarian stimulation with a standard gonadotropin-releasing hormone antagonist protocol. The public fertility clinics at the Copenhagen University Hospitals in Herlev and Hvidovre, Stork IVF clinic and the fertility clinic at Skåne University Hospital included patients in the study.
Participants/materials, setting, methods: MGC were obtained from women with varying ovarian reserve status (defined from age dependent anti-Müllerian hormone (AMH) levels) by isolation from pooled follicles immediately after oocyte retrieval. DNA from the MGC aggregates was extracted and analysed with the Illumina EPIC array, at the Human Genotyping Facility Genetic Laboratory, Dept. of Internal Medicine Erasmus MC Rotterdam. Subsequently, the data went through a bioinformatic analysis. All analysis included adjustment for chronological age.
Main results and the role of chance: None of the epigenetic clock tools that were applied showed an association between age acceleration and ovarian reserve in either cell type. Performing a differential DNA methylation variability analysis by comparing DOR or high ovarian reserve samples to controls (normal ovarian reserve), differential variability between DOR and normal samples was observed at 4,199 CpGs in MGC, while only 447 between high and normal (FDR < 0.05). Regardless of ovarian reserve, very few signals were detected in leukocytes and no overlaps with the signals in MGC were found. Several genes central to folliculogenesis, i.e. inhibin subunit beta B (*INHBB), AMH,* insulin-like growth factor 2 (*IGF2*), were associated with variably methylated CpGs. Gene ontology analysis of genes associated with the variable CpGs in DOR in MGC revealed an enrichment for categories related to cell-to-cell adhesion ($p<0.001$). Furthermore, we found a higher number of epimutations in the MGC from women with DOR.
Limitations, reasons for caution: The granulosa cells were collected during ovarian stimulation which may influence DNA methylation. We were not able to correct for the FSH dose. However, we have previously shown no association between FSH dose and DNA methylation profile.
Wider implications of the findings: Clarification of a potential unstable methylome and epigenetic dysfunction in MGC being associated with the poor ovarian reserve found in women with DOR may be important for future treatment and prevention strategies.
Trial registration number: not applicable

P-533 Preferential allelic segregation of DNA triplet repeat expansion on PGT-M embryos from Myotonic Dystrophy type 1 and Huntington´s disease

P. Villa[1], R. Quintana[2], Q. Ramiro[3], M. Sandra[4], J.V. Martinez-Sanchis[5], M. De la Orden[5], I. Moya[5], T. García-Gimeno[3], A. Monzo[3]

[1]La Fe University Hospital-Joint First authors, Women's Health Area, Valencia, Spain ;
[2]La Fe University Hospital-Joint First authors, Women's Health Area, Valencia, Spain ;
[3]La Fe University Hospital, Human Reproduction Unit- PGD-Unit, Valencia, Spain ;
[4]La Fe University Hospital, Department of Genetics, Valencia, Spain ;
[5]La Fe University Hospital, Human Reproduction Unit. IVF Lab, Valencia, Spain

Study question: Are there any differences in parental origin of triplet repeat expansion in human embryos after PGT-M from Myotonic Dystrophy type 1 or Huntington's Disease carriers?
Summary answer: Triplet repeat expansion mutations in DMPK and HTT genes have a preferential gender pattern of transmission to embryo yield.
What is known already: Myotonic dystrophy 1 (MD1) and Huntington's disease (HD) are progressive autosomal dominant neurodegenerative disorders caused by a CGG or CAG repeat expansion in the *DMPK* or *HTT* genes, respectively. Preimplantation genetic testing (PGT) is a diagnostic procedure available for these individuals, because they carry a higher risk of transmitting this genetic condition to their offspring. It has been proposed preferential expansion depending on the gender parental carrier.

Study design, size, duration: Information about 82 PGT-M cycles performed from 2011 to 2019 in 14 MD1 and 18 HD couples was collected retrospectively. MD1: 21 PGT-M cycles in 9 female-carrier and 9 cycles in 5 male-carrier couples. HD: 39 cycles in 14 female-carrier and 13 cycles in 5 male-carrier couples.
Participants/materials, setting, methods: All procedures were performed at the Human Reproduction and Genetic Units in a tertiary University Hospital. PGT provided direct testing of embryos obtained after intracytoplasmic sperm injection, using polymerase chain reaction PCR of microsatellite markers (indirect method) and by expansion amplification (direct method) as the genetic testing protocol to detect the risk allele and the expanded CTG/CAG repeat in 366 day 3 embryos, 167 from MD1 and 199 from HD.
Main results and the role of chance: The percentage of non-affected embryos related to the parent-of-origin in DM1 was 35.5% (43/121) in female carriers and 19.6% (9/46) in male carriers. PGT-M in HD resulted in 39.6% (67/169) in female carriers and 16.7% (5/30) in male carriers.
In both conditions, we observed significantly higher ratio of non-affected embryos in female carriers when compared to male carriers. (38%: 110/290 vs 18.4% (14/76), respectively. Chi square test P=0.026).
Of the total sample, 38 embryos (10.4%) were misdiagnosed, mainly due to allele drop out (ADO) or amplification failure.
Limitations, reasons for caution: This study has a retrospective design. The number of patients is far low in some groups, although the number of embryos is adequate for statistical tests. Misdiagnosed embryos has a potential to bias results.
Wider implications of the findings: In both conditions, the observed non-affected embryos rate is far below of the theorical 50% expected, according to autosomal dominant inheritance. Our results must be taken into account in order to offer the appropriate genetic counseling in carrier patients before PGT-M.
Trial registration number: Not applicable

P-534 Probability of producing at least one healthy transferable cleavage-stage embryo after preimplantation genetic testing for fragile X syndrome

C. Sonigo[1], M. Sadoun[1], L. Hester[2], J. Steffann[3], A. Benoit[1], V. Gallot[1], N. Frydman[2], M. Grynberg[1]

[1]Antoine Béclère Hospital, Reproductive Medicine and fertility preservation, Clamart, France ;
[2]Antoine Béclère Hospital, reproductive biology unit, Clamart, France ;
[3]Necker Enfants Malades Hospital, Genetic Unit, Paris, France

Study question: What are the chances of obtaining a healthy transferable cleavage-stage embryo according to the number of mature oocytes in *FMR1*-mutated carriers undergoing preimplantation genetic testing?
Summary answer: Fifteen mature oocytes provide 69% chance of obtaining at least one healthy embryo after preimplantation genetic testing (PGT) in *FMR1*-mutated carriers.
What is known already: PGT may be an option to achieve a pregnancy with a healthy baby for *FMR1* mutation carriers. In addition, *FMR1* premutation is associated with a higher risk of diminished ovarian reserve and premature ovarian insufficiency. As a consequence, oocytes vitrification may be offer to these young women as a method of fertility preservation. The cryopreserved oocytes can then be used to achieve pregnancy, with or without PGT. The number of vitrified oocytes to allow the transfer of a healthy embryo following PGT has never been investigated.
Study design, size, duration: This is a monocentric retrospective observational study analyzing 33 premutated (n=17) or fully mutated (n=16) candidates for PGT for fragile X syndrome between 2006 and 2018.
In the meantime, 15 *FMR1* premutated carriers underwent oocyte vitrification for fertility preservation (FP).
Participants/materials, setting, methods: Eighty-five PGT cycles were performed for fragile X syndrome, 43 in premutated patients and 42 in fully mutated women. For each cycle, we estimated the number of mature oocytes for obtaining at least one healthy embryo after PGT using Visual Basic for Applications (VBA) analysis.
Main results and the role of chance: Overall, the total number of retrieved and mature oocytes were 12.1 ± 6.1 and 9.4 ± 5.2 respectively. Among the 7.2 ± 4.6 day-3 embryos obtained, a mean number of 3.4 ± 3.0 embryos were morphologically eligible for biopsy, leading to 1.5 ± 1.6 healthy embryo on average. The obtaining of 3 mature oocytes were associated with 28.6% chance of having at least one healthy embryo. This probability increased significantly with the number of mature oocytes. Ten mature oocytes led to more than 60% chance of obtaining a healthy embryo. This probability did not exceed 69% and plateaued from 15 metaphase II oocytes collected. In the FP group, the mean number of mature oocytes vitrified was 5.1 ± 3.6 per FP cycle. On average, 3 cycles of ovarian stimulation were performed per patient leading to a mean of 15 mature oocytes vitrified**.**
Limitations, reasons for caution: This study is retrospective, analyzing a limited number of cycles, without possibility to determine threshold values of oocytes that might predict the number healthy embryo.
Wider implications of the findings:
The present results should be confirmed by further analyzes. Patients included in a fresh PGT cycle usually show higher values of ovarian reserve tests when compared with *FMR1* premutated carriers seeking FP. Whether the oocyte competence differs in these groups of patients is unknown. This information might balance our conclusion.
Trial registration number: not applicable

P-535 Antral follicle responsiveness to exogenous FSH is not altered in FMR1 mutation carriers undergoing stimulation for preimplantation genetic testing.

M. Grynberg[1], M. Pinto[1], A. Naouel[1], V. Gallots[1], A. Mayeur[2], C. Sonigo[1]

[1]Hôpital Antoine Béclère, Reproductive Medicine & Fertility Preservation, Clamart, France ;
[2]Hôpital Antoine Béclère, Reproductive biology unit, Clamart, France

Study question: Is antral follicle responsiveness to exogenous FSH, assessed by the follicular output rate (FORT), altered in *FMR1*-mutation carriers?
Summary answer: FMR1-mutation carriers do not show alteration in antral follicle responsiveness to exogenous FSH assessed by the FORT index
What is known already: Female carrying *FMR1* mutation (premutation or full mutation) may be candidate to preimplantation genetic testing (PGT) to achieve a pregnancy with a healthy baby while avoiding the burden of prenatal genetic diagnosis and the risk of pregnancy termination. Several lines of evidence indicate that female carrying *FMR1* premutation are at higher risk of diminished ovarian reserve and further premature ovarian insufficiency. However, whether this genetic profile alter the capacity of small antral follicle to respond to exogenous FSH during controlled ovarian stimulation remains unelucidated.
Study design, size, duration: Monocentric retrospective observational study. Thirty-three patients carrying either *FMR1* premutation (n=17) or full mutation (n=16) were referred to our PGT centre from 2006 to 2018.
Participants/materials, setting, methods: Eighty-five PGT cycles for fragile X syndrome, 43 in premutated patients and 42 in fully mutated women, were analyzed. COS outcomes were compared to 85 PGT cycles performed for male translocation, matched on female age, BMI, antral follicle count, and protocol of stimulation. FORT was determined by the ratio between the pre-ovulatory follicle count (PFC, 16-22 mm) on the day of oocyte triggering × 100/ antral follicle count measured just before initiation of the stimulation.
Main results and the role of chance: By design, patients were comparable in terms of age, BMI and markers of the follicular ovarian status. Overall, after similar starting dose (253±91 vs. 247±82, p=0.7 and total dose of gonadotropin administered (2623±1071 vs. 2620±1029 IU, p=0.98), the numbers of oocytes recovered and mature oocytes (12.1±6.1 vs. 11.8±4.9, *p=0.70;* and 9.4±5.2 vs 9.9±4.1; *p=0.70,* respectively) were not significantly different in *FMR1*-mutation carriers and controls. In addition, FORT index remained similar between both groups (42.6±23.9% *vs* 42.4±27.2%, *p=0.96*, respectively).

Comparison of *FMR1* premutation and full mutation carriers showed lower values of ovarian reserve tests in permutated patients (AMH 3.5±3.2 *vs.* 6.2±3.6 ng/mL, p=0.0004 and AFC 18.7±9.4 *vs.* 22.9±8.9 follicles, p=0.03). Therefore, significantly higher doses of total FSH were needed (2988±1184 vs. 2256±867 IU, p=0.002) to obtain the similar oocyte yield (12.2±6.6 *vs.* 11.9±5.7, p=0.8) and number of mature oocytes (12.2±6.6 *vs.* 11.9±5.7, p=0.8). The FORT index was significantly higher in *FMR1* premutation carriers when compared to full mutation carriers (48.8±26.5 *vs.*37.4 ±19.5%, p=0.03).
Limitations, reasons for caution: This study is retrospective, analyzing a limited number of cycles. In addition, women included in a PGT program do not

reflect the whole population of *FMR1*-mutation carriers. Indeed, those with the lowest values of ovarian reserve tests are often not eligible for PGT.
Wider implications of the findings: *FMR1*-mutated carriers eligible for PGT may not require specific adjustments in their stimulation protocol. Further analysis is required to confirm these data on a larger population.
Trial registration number: not applicable

P-536 Difference in aneuploidy rates between embryos biopsied on day 5, 6 and 7, cultured in medium with and without granulocyte-macrophage colony-stimulating factor (GM-CSF)

A. Bruszczynska[1], M. Rusin[2], M. Fidzianska[2], M. Jagiello[2], A. Wozniak.[1], J. Liss[1,3], A. Knight[1], K. Lukaszuk[1,4,5]

[1]Invicta, Fertility and Reproductive Centre, Gdansk, Poland ;
[2]Invicta, Fertility and Reproductive Centre, Wroclaw, Poland ;
[3]University of Gdansk, Department of Medical Biology and Genetics, Gdansk, Poland ;
[4]Medical University of Warsaw, Department of Gynecological Endocrinology, Warsaw, Poland ;
[5]Medical University of Gdansk, Department of Obstetrics and Gynecological Nursing- Faculty of Health Sciences, Gdansk, Poland

Study question: Are aneuploidy rates different for embryos qualified for trophectoderm biopsy on day 5, 6 or 7 of culture, in medium with or without GM-CSF?
Summary answer: PGT-A results showed an increasing proportion of abnormal blastocysts on days 6 and 7 comparing to day 5 but not difference for cultures with/without GM-CSF.
What is known already: Chromosome analysis is performed to ensure that embryo transferred to the patient has a correct number of chromosomes to reduce the risk of abnormal pregnancy, miscarriage and failed implantation. Granulocyte-macrophage colony-stimulating factor has a beneficial effect on the development of human embryos in assisted reproductive technology (ART).
Study design, size, duration: This is a retrospective study performed using the medical records of patients who underwent IVF procedures with PGT-A between January 2017 and December 2019 at INVICTA Fertility Clinic, Poland. 1854 embryos cultured to a blastocyst stage were examined for aneuploidy. Trophectoderm biopsy was performed on day 5 of development or day 6 and 7 for slower growing embryos. Embryo culture medium was selected based on the clinicians and patients decision.
Participants/materials, setting, methods: Analysis included 655 treatment cycles. Median patient age was 37 (interquartile range: 33-39). Embryos were qualified for trophectoderm biopsy on days: 5, 6 or 7 depending on their development. PGT-A was performed using next generation sequencing (NGS). Additionally, the collected data was classified according to the type of culture medium – with GM-CSF (EmbryoGen/BlastGen, Origio) or without (G1 PLUS/G2 PLUS, Vitrolife).
Main results and the role of chance: Among the 1854 analyzed embryos, 584 were cultured in medium containing GM-CSF. In that group 229 embryos were qualified for biopsy on day 5. 68.1% of them were euploid and 31.9% aneuploid. On day 6, material was collected from 342 embryos - 52.6% euploid. On day 7, among 13 examined embryos and 23.1% were euploid. The differences in proportions of euploid embryos biopsied on different days were shown to be statistically significant ($p<0.001$).
Among the 1270 embryos cultured in the standard medium (i.e., without GM-CSF), on day 5, biopsy was performed on 405 embryos and 64.2% of them were euploid. On day 6 – 55.9% were normal, on day 7 – 50.0%. This comparison showed that with each next day, the percentage of embryos with normal PGT results decreases. The differences in proportions of euploid embryos were statistically significant ($p<0.001$).
There also appears to be a linear trend in the decrease of euploidy rate with each additional day needed to the embryo to reach qualification for biopsy (GM-CSF: $p<0.001$, no-GM-CSF: $p<0.001$).
No significant difference was observed in general percentage of correct result between embryos cultured with or without GM-CSF (58,1% vs 59,9%).
Limitations, reasons for caution: The possible limitation is that embryo culture medium selection was not randomized but rather based on medical indications (outcomes of previous cycles) and also on financial resources of the patients, as GM-CSF supplementation was more expensive for the patient.
Wider implications of the findings: Presented results suggest that addition of GM-CSF does not change the general percentage of euploid embryos. Although no benefit of GM-CSF on percentage of chromosomally normal embryos was observed in this study, there is a need for further randomized studies that could correlate findings with other factors.
Trial registration number: not applicable

P-537 Adopting machine learning (ML) and artificial intelligence (AI) methods to minimize the use of invasive biopsy techniques in embryo assessment and selection

N. Zaninovic[1], J. Sierra[2], J. Barnes[3], P. Khosravi[3], I. Hajirasouliha[3], Q. Zhan[1], J. Malmsten[1], Z. Rosenwaks[1]

[1]Weill Cornell Medicine, The Center for Reproductive Medicine, New York- NY, U.S.A. ;
[2]The Manhattan College, Computational Biology, New York, U.S.A. ;
[3]Weill Cornell Medicine, Department for Precision Medicine, New York- NY, U.S.A.

Study question: Can an AI-informed technique be developed to select embryos with a high probability of euploidy and indicate when more invasive methods (PGT) should be adopted?
Summary answer: Incorporating AI-ML methodology for embryo assessment results in a high-fidelity embryo ploidy prediction model that can provide guidance when more invasive assessment techniques are required.
What is known already: Selecting the best embryo for transfer is an enduring objective in IVF treatments. Embryo grading and time-lapse technology have helped to determine key attributes of embryo quality that correlate to clinical outcomes. The development of a noninvasive method of embryo screening as an alternative to invasive and expensive PGT is vital.
Study design, size, duration: This is a retrospective study of blastocysts (N=10491) of known ploidy status with embryo morphokinetic and morphology annotations and associated patient clinical profiles.
Participants/materials, setting, methods: Patients undergoing their first or second ICSI cycle using PGT were included. All embryos were cultured with a time-lapse (TLM) system. Variables studied included the morphokinetic timings, TLM images of the embryos, and blastocyst grades of the ICM, trophectoderm, and expansion. Ploidy was determined by PGT methods conducted on-site.
Main results and the role of chance: Multiple ML approaches and AI (deep learning) algorithms were implemented to determine the most expedient and reproducible method to provide a reliable assessment of embryo ploidy. To address the role of chance, 80:20 bootstrapping methods were used to develop ranges in the model parameters and predictions. The area under the receiver operating curve (AUC) obtained from the ML model was 0.734. This model, using a preset threshold, was able to detect 25% of blastocyst ploidy status with high accuracy (95% pos. and 94% neg.) that an invasive biopsy was not necessary. For the remaining 75% of the population, the model estimates could be incorporated into other clinical information to determine the acceptable level of risk to undertake an invasive biopsy. The deep learning model, trained on embryo images of blastocysts at 110 hours, showed an accuracy of 62.5%. The addition of embryological information improved the accuracy of the model to 70.4%.
Limitations, reasons for caution: The single-center and retrospective nature of the analysis could be a limiting factor, although the findings were validated through a large dataset.
Wider implications of the findings: The implementation of AI/ML algorithms trained on a large data set of blastocysts of known ploidy suggests that noninvasive approaches may be used to assess embryos with a high level of confidence. These techniques may also help us to identify embryos for which PGT is recommended.
Trial registration number: not applicable

P-538 Pericentromeric DNA transcripts are involved in the formation of membraneless mitochondria-associated structures at the end of oocyte maturation in human..

N. Enukashvily[1], A. Dobrynin[1], N. Korchagina[2], A. Prjibelski[3], D. Shafranskaya[4]

[1]Institute of Cytology- RAS, The Non-Coding DNA Lab, St. Petersburg, Russia C.I.S. ;
[2]Ava-Peter-Scandinavia Clinics, Embryology Dept, st. Petersburg, Russia C.I.S. ;
[3]St. Petersburg State University, Center for Algorithmic Biotechnology, St.Petersburg, Russia C.I.S. ;
[4]St. Petersburg State University, Biology Dept, St.Petersburg, Russia C.I.S.

Study question: Whether maternal long non-coding RNAs (lncRNAs) transcribed from pericentromere non-coding tandemly repeated (TR) DNA are involved in the formation of membraneless structures in GV-MI oocytes.
Summary answer: In late preovulatory oocytes, pericentromere tandemly repeated DNAs are transcribed. The transcripts are clustered in some membraneless RNP structures containing helicases and associated with mitochondria.
What is known already: Tandemly repeated DNA (TR DNA) constitutes approximately 10% of the human genome. TR DNA family includes microsatellites, minisatellites, and satellites. Classical human satellites 1, 2, 3 (HS1, HS2, HS3) underlie the pericentromere region. Pericentromeric satellites transcripts play an important role in mammalian embryogenesis. Transcripts of satellite DNA are present in mouse embryos prior to zygotic activation. However there are no data about pericentromeric TR RNA in human oocytes.

In mammals, pericentromeric transcripts are involved in assembling membraneless bodies. However, in mammalian oocytes membraneless bodies enriched in non-coding RNAs and helicases are believed to be disassembled in the meiosis I prophase.,
Study design, size, duration: GV and MI donor oocytes are not used for gametes banking and are usually discarded. They were donated for scientific purposes after the local ethic committee permission was granted and a donor informed consent was signed.
The HS 2,3 transcripts distribution, their colocalisation with helicases and mitochondria were studied in GV (n=12), MI (n=8) oocytes. The presence of HS2,3 transcripts in published transcriptomes of healthy donors was verified with computational methods.
Participants/materials, setting, methods: The HS 2, 3 transcripts distribution was studied with RNA-DNA FISH. The colocalisation of the transcripts with helicases was estimated by immunoFISH. Double immunostaining was used to study the spatial relationship of helicases and the import receptors Tom20 of mitochondrial preprotein translocases of the outer membrane (Tom). The presence of HS2,3 transcripts in published transcriptomes of healthy donors was verified with computational analysis of published healthy donors transcriptomes
Main results and the role of chance: HS2, 3 transcription increased in in oocytes after the GV stage. In GV oocytes, the intranuclear DDX5 helicase was adjacent to the heterochromatic ring surrounding the post-nucleolar body. In late GV-MI and in MI, the HS2, 3 transcripts were colocalised with the helicases granules that overlapped with the oocyte mitochondria. In published transcriptomes, several polyadenylated transcripts from different datasets were detected. All the transcripts belonged to HS TR DNA families. The cDNAs had the typical signatures of HS sequences. One of transcripts contained the sequence we previously identify in transcriptomes of cancer and fetal cells.
Thus, in late preovulatory oocytes, different HS families of TR DNA are transcribed. The transcripts are clustered in some membraneless RNP structures that contain helicases and are closely associated with mitochondria being therefore similar to Balbiany bodies described previously in early mammalian oocytes. To date no membraneless granules associated with mitochondria were described in oocytes at the late stages of maturation. Mitochondria associated membraneless coacervate bodies assembled on the base of pericentromeric transcripts might be a place for such spatial sequestration in the same way as in early oocytes.
Limitations, reasons for caution: The experiments were performed on fixed cells. The in vivo visualisation of the structures will be carried out to prove the existence of the granules in a living oocyte
Wider implications of the findings: Transcription of HS2,3 occurs in late maturing oocytes that were assumed to be transcriptionally silent. The existence of membraneless bodies was demonstrated for the first time. The obtained results give a new perspective to study the late oogenesis.
Trial registration number: not applicable

P-539 Transcription of human pericentromeric non-coding DNA at the end of human oocyte maturation

M. Dobrynin[1], A. Prjibelski[2], N. Korchagina[3], D. Shafranskaya[4], N. Enukashvily[1]

[1]Institute of Cytology of Russian Academy of Sciences, Laboratory non-coding DNA, St. Petersburg, Russia C.I.S. ;
[2]St. Petersburg State University, .Center for Algorithmic Biotechnology, St. Petersburg, Russia C.I.S. ;
[3]Ava-Peter – Scandinavia ART Clinic, Clinic on Nevsky prospect, St. Petersburg, Russia C.I.S. ;
[4]St. Petersburg State University, Faculty of Biology, St. Petersburg, Russia C.I.S.

Study question: Whether maternal long non-coding RNAs (lncRNAs) transcribed from pericentromere non-coding tandemly repeated (TR) DNA are accumulated in late maturating oocyte
Summary answer: The maternal transcripts of pericentromere TR DNA are accumulated in the late oocyte maturation. They are present in transcriptomes of MI, MII oocytes.
What is known already: Non-coding RNAs play an important role in mammalian gameto- and embryogenesis. Human pericentromere is built on TR DNA of "classical" satellites family - human satellite (HS) 1, 2, 3 (HS1, HS2, HS3). Blocking the pericentromere TR DNA transcription leads to embryo development arrest in mouse. Transcripts of TR DNA are present in mouse embryos prior to zygotic activation in small quantities suggesting they are not only transcribed in embryo but are accumulated in maternal ooplasm. However there are no data about pericentromeric TR DNA and their transcripts in human oocytes.
Study design, size, duration: We analysed 8 RNA-Seq published datasets of healthy donors MI and MII oocytes obtained by 4 independent research groups. The biocomputational analysis results were verified by fluorescence in situ hybridization (FISH). FISH was carried out on MI human preovulatory oocytes without morphological abnormalities from healthy donors. These oocytes were excluded from egg donation program and were used in the present study if an informed consent was signed by a donor.
Participants/materials, setting, methods: Raw reads were quality- and adapter-trimmed in order to check the presence of sequences containing homology regions with the HS2,3 probe. Only transcripts containing highly confident matches with HS2,3 were selected . Several polyadenylated transcripts were detected (length 300-640 bp). They were analyzed for regions of similarity, multiple sequence alignment. The same probe as in transcriptome analysis was used as a DNA-RNA FISH probe.
Main results and the role of chance: All four selected transcripts belonged to pericentromere TR DNA families. Multiple alignment of all the 4 transcripts and pairwise alignment of sequences revealed that they are not identical and belong to different families of classical satellites. All the assembled transcripts shared homology (identity more than 75%) with different satellite lncRNA previously obtained by from heat-shocked HeLa cells. One of the transcripts we assembled shared high degree homology (> 80%) with the boundary region between centromeric DNA and pericentromeric HS. Moreover, the same transcript contained the complete HS3 sequence that we had found earlier transcribed in some cancer cell lines, senescent lung fibroblasts and some tissues of a developing human embryo. The transcripts revealed by computational analysis were detected in MI oocytes by RNA-FISH. The transcripts were associated in granules scattered throughout the ooplasm.
Limitations, reasons for caution: The sample size for FISH analysis in this study was small due to difficulties in obtaining human preovulatory oocytes without morphological abnormalities. Further studies, including analysis of transcription of HS2,3 dynamics by live-cell imaging, may help define the role that perform of these transcripts in oocyte maturation and early embryogenesis.
Wider implications of the findings: According to our data, transcription of HS2,3 occurs in late maturing oocytes that were assumed to be transcriptionally silent. The appearance of HS3 transcripts in the MI oocyte suggests that these transcripts might be important for oocyte maturation or early embryogenesis.
Trial registration number: not applicable

P-540 correcting a PLCζ mutation in the human germ line to overcome hereditary infertility

B. Bekaert[1], A. Boel[1], M. Popovic[1], P. Stamatiadis[1], R. Reddy Guggilla[1], S. Chuva de Sousa Lopes M. C.[2], P. De Sutter[1], P. Coucke[3], B. Heindryckx[1]

[1]UGhent, Department of Human structure and repair, Ghent, Belgium ;
[2]Leiden University Medical Center, Department of Anatomy and Embryology, Leiden, The Netherlands ;
[3]UGhent, Department of Biomolecular Medicine, Ghent, Belgium

Study question: Can clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing be used for correction of a single base pair substitution in phospholipase C zeta (PLCζ)?
Summary answer: CRISPR/Cas9 administration during ICSI enables the correction of a single base pair substitution in PLCζ, possibly by interhomologue homologous recombination.
What is known already: Mutations in the *PLCZ1* gene, encoding for phospholipase C zeta (PLCζ) -the sperm-related oocyte activating factor- can result in failed fertilization after ICSI, which can be overcome with assisted oocyte activation (AOA). However, children may still inherit the disease-causing mutation resulting in the need for future reproductive treatment. A possible method to overcome mutation transmission is gene correction with CRISPR/Cas9 during ICSI. From previous studies using CRISPR/Cas9 in the human germline, it is still called into question whether gene correction predominantly occurs through interhomologue homologous recombination or the use of the added repair template.
Study design, size, duration: We aimed to correct a failed fertilization-related paternal mutation by delivering CRISPR/Cas9 components during ICSI. A gRNA-Cas9 protein complex specifically designed to target the mutant allele, and a repair template harboring the desired base pair substitution and an additional synonymous mutation, were injected together with patient's sperm. AOA was performed following ICSI to overcome fertilization failure. Fertilized zygotes were cultured for 3-5 days. These reconstructed embryos (n=12) were thoroughly analyzed to assess gene editing efficiency.
Participants/materials, setting, methods: We recruited one male patient with a heterozygous base pair substitution in *PLCZ1* (c.136-1G>C) leading to failed fertilization in all oocytes after routine ICSI. Donated *in vitro* or *in vivo* matured oocytes containing clusters of smooth endoplasmic reticulum were targeted, next to a control group without gene editing. Targeted next-generation sequencing was used to assess correction potential and short tandem repeat (STR) analysis to characterize whether mutation-carrying or wild-type sperm gave rise to the embryo.
Main results and the role of chance: In the control experiments where no CRISPR/Cas9 was added, ICSI with the patient's sperm and AOA treatment, resulted in a mutation distribution of approximately 50%. High specificity of the designed CRISPR/Cas9 components for targeting the paternal mutant *PLCZ1* allele was demonstrated by the absence of unspecific insertions/deletions (indels) in embryos originating from wild-type sperm (n=5). When the mutant *PLCZ1* allele was observed, indicated by the presence of the mutant chromosome-related STR markers, either no editing (n=3), additional mutagenesis (indels) (n=2) or correction (n=2) occurred. Remarkably, our results suggest that the correction process may not require the presence of a repair template, due to the absence of the synonymous mutation incorporated into the repair template in the DNA sequence of the corrected embryos. In addition, for the STR markers close to the Cas9 cut site only one novel (presumably the maternal) allele was present, while both maternal and paternal alleles were identified for STR markers further upstream and downstream from the Cas9 cut site. This suggests that the correction resulted from interhomologue homologous recombination, by which the maternal wild-type allele is used as a template to repair the double-strand break.
Limitations, reasons for caution: One of the major limitations of CRISPR/Cas9 is the occurrence of mosaicism and off-target editing, which still remains to be evaluated. Moreover, the number of targeted embryos should be increased to more reliably estimate the accuracy and efficiency of the CRISPR/Cas9 protocol.
Wider implications of the findings: Our findings demonstrate that CRISPR/Cas9 may serve to correct heritable infertility mutations in the germ line. Our results further confirm the occurrence of interhomologue homologous recombination, which may lead to loss of heterozygosity. Additional studies should be undertaken to evaluate the safety/efficiency of this method prior to clinical applications.
Trial registration number: not applicable

P-541 Immunostaining of global 5mC and 5hmC levels of DNA in differentially protaminated human sperm chromatin

M. Olszewska[1], O. Kordyl[1], M. Kamieniczna[1], M. Fraczek[1], P. Jedrzejczak[2], M. Kurpisz[1]

[1]Institute of Human Genetics- Polish Academy of Sciences, Poznan, Poland ; [2]Poznan University of Medical Sciences, Department of Infertility and Reproductive Endocrinology, Poznan, Poland

Study question: To determine the relationship between 5mC and 5hmC according to the sperm chromatin protamination, in 3 sperm populations: normally protaminated, less protaminated, and deprotaminated, in fertile and infertile males
Summary answer: High DNA 5mC level is a marker for well-protaminated spermatozoa, documenting proper spermatogenesis. 5mC/5hmC levels are highest in properly-protaminated spermatozoa, but disrupted in oligoasthenozoospermia
What is known already: A special role in etiology of male infertility play epigenetic modifications, including methylation (5mC) and hydroxymethylation (5hmC) of DNA. Global DNA methylation level is high in normozoospermia, which confirms a correct spermatogenesis. Published data also indicate the link between sperm quality and the protamination state of sperm chromatin.
Study design, size, duration: 50 patients with oligo-/oligoasthenozoospermia and reproductive failure (P group) vs. 30 normozoospermic controls (K group). Both groups: normal karyotype (46,XY), 25-30 years old, and a lack of smoking habits, any stimulants/drugs usage, and toxic work environment.
Participants/materials, setting, methods: For the first time, a sequential staining protocol was applied, which allowed to analyze the 5mC/5hmC levels revealed by immunofluorescent (IF) stainings using the same spermatozoa with previously determined chromatin protamination status (aniline blue staining). Also TUNEL examination was performed to check the sperm DNA fragmentation status.
Main results and the role of chance: In the P group, both: the protamination level of sperm chromatin, as well as 5mC and 5hmC levels were decreased, when compared to the K group. Results obtained in P group also pointed out a higher interindividual heterogeneity. The 5mC and 5hmC levels were diversified in respect to the chromatin protamination status: protaminated sperm cells showed the highest 5mC and 5hmC values, in both: K and P groups. A negative correlation was found between 5mC level vs. correct protamination (both groups of males; $p<0.05$). Negative correlation was also identified for 5hmC level vs. correct protamination in the K group, in contrast to the P group, where 5hmC level increased concurrently with the chromatin protamination status ($p<0.05$). Sperm DNA fragmentation was higher in the P group vs. K group.
Limitations, reasons for caution: Analyzes on bigger number of patients are required
Wider implications of the findings: Measuring of 5mC/5hmC status of sperm DNA evidences the correctness of spermatogenesis and its disruption may be a marker of reproductive failure.
Study funding/competing interest(s)
2015/17/D/NZ5/03442, National Science Centre in Poland. Authors declare no competing interests.
Trial registration number: na

P-542 Transfers of chromosomal-abnormal "mosaic" and "aneuploid" embryos in patients previously refused such transfers at centers were embryos had been produced

D. Barad[1], D. Albertini[2], E. Molinari[2], N. Gleicher[1]

[1]Center for Human Reproduction, n/a, New York, U.S.A. ; [2]Center for Human Reproduction, Laboratories, New York, U.S.A.

Study question: What are outcomes of transfers of chromosomal-abnormal embryos ("mosaic" and "aneuploid") after patients were refused such transfers by their IVF centers, where those embryos were established?
Summary answer: Transfers of such embryos result in significant life birth rates with basically no risk for births of chromosomal-abnormal offspring.
What is known already: Like earlier studies, the STAR study recently again failed to demonstrate outcome benefits from chromosomal testing of embryos prior to transfer (PGT-A). Moreover, hundreds of chromosomal-normal offspring have been reported born following transfer of chromosomal-abnormal embryos ("mosaic" and "aneuploid"), without even a single chromosomal-abnormal birth. Yet, PGT-A practice continues, and most IVF centers still refuse transfers of embryos, by PGT-A declared "mosaic" and/or "aneuploid."
Study design, size, duration: Prospective observational study of 50 patients who moved 278 allegedly chromosomal-abnormal embryos (per PGT-A) between June 2016 and October 2019 to our center after having been refused transfer of some of these embryos at the centers where those embryos had been produced. The center's experience preceding 2016 has been reported before.

Participants/materials, setting, methods: PGT-A was performed by NGS or CGH. Consents pointed out risks of chromosomal-abnormal pregnancies. Without evidence for a 80% threshold of "aneuploid" DNA between "mosaic" and "aneuploid" biopsies, and no differences in reported outcomes, we do not differentiate between "mosaic" and "aneuploid" embryos. Transfers are recommended in absence of 3 or less known "survivable" chromosomal abnormalities. Patients are, however, entitled to override. Consents since 2014 oblige to early prenatal diagnostics and termination with aneuploidies.
Main results and the role of chance: 32 patients underwent transfers (age 40.6 ± 4.1 years); 31/32 (96.9%) had at least one previous IVF cycles (total, n=176); 20/32 (62.5 %) had at least one prior pregnancy (overall, n=40); but only 6/32 (18.8%) prior live births. In 32 transfers, 101 embryos were placed (median 3; range 1-5), 74 embryos were mosaic, 19 aneuploid and 8 undetermined. Three live births and two ongoing pregnancies beyond 20 weeks (total likely live birth rate 5/32 (15.6%) were established; 4 pregnancies were miscarried (total pregnancy rate 28.1%), 2 concordant with PGT-A results, 1 discordant (normal 46,XX - the patients had SLE), and 1 patient refused testing.
Limitations, reasons for caution: At mean age 40.6, patients in this study are significantly older than in any previously reported patient population receiving "abnormal" embryos. Here presented data, therefore, likely underestimate pregnancy and live birth outcomes and overestimate miscarriage rates for younger women.
Wider implications of the findings: Transfer of selected, by PGT-A designated to be chromosomal-abnormal embryos, whether "mosaic" or "aneuploid," under current reporting standards is safe, and results in delivery of significant chromosomal-normal offspring which never would be born if these embryos had not been transferred.
Trial registration number: n/a

P-543 A whole genome analysis for simplified a preimplantation genetic testing of a rare and a complex interchromosomal reciprocal insertion: thorough investigations for a straightforward interpretation.

R. Zenagui[1], I. Bernicot[1], T. Anahory[1]

[1]Montpellier Hospital, PGT depatment, Montpellier, France

Study question: Should whole genome sequencing (WGS) be considered for the diagnosis of complex chromosomal abnormalities before a preimplantation genetic testing of structural rearrangement (PGT-SR) attempt?
Summary answer: WGS should be considered to establish the complexity of chromosomal abnormalities with accuracy before PGT-SR investigations.
What is known already: Insertions are rare events and interchromosomal reciprocal insertion (IRI) with four breakpoints are exceptional, with only 11 cases reported to date. To the best of our knowledge, no preimplantation genetic testing for structural rearrangement (PGT-SR) was performed to investigate the management of patients with IRI abnormalities.
Study design, size, duration: A male carrier of rare IRI 46,XY,ins(14;22)(q11.2 ;q11.2) and his partner were directed to our center for PGT analysis after three spontaneous miscarriages. The patients were informed of the investigations and gave their consent before participation in the study.
Participants/materials, setting, methods: A genome sequencing of a male carrier was conducted at the French National Research Centre for Human Genomics. Library generation was performed with TruSeq® DNA PCR-free kit. The genome was sequenced on a HiSeq X5 following the manufacturer's instructions. DNA sequences were mapped to the reference human genome sequence (GRCh37). Structural variations were called using MANTA. FISH assays were used to detect all chromosome segments involved in chromosomal rearrangement and to identify transferred normal/balanced embryos.
Main results and the role of chance: Whole genome sequencing analysis allowed to determine accurately the chromosomal breakpoint numbers between the chromosome 14 and the chromosome 22. Against all odds, two breakpoints were identified instead of four as expected in IRI rearrangements. The junction fragments sequencing confirmed the location and the number of both breakpoints. Finally, the patients were informed that the chromosomal abnormality was a reciprocal translocation instead of interchromosomal reciprocal insertion. Thereby, a FISH probe strategy was adapted to highlight all chromosomal imbalances, PGT-SR attempt was achieved and a healthy baby was born.
Limitations, reasons for caution: No FISH probes could be established between the centromeres and the chromosomal breakpoints on either or both involved chromosomes. This limitation may lead to a misintepretation of breakpoints number affecting chromosomes.
Wider implications of the findings: FISH techniques aren't capable of identifying all complex chromosomal rearrangements, mainly those involving centromeric regions of acrocentric chromosomes, investigations of those abnormalities should be performed with a sensitive methods.The advent of new technologies allowed better appreciations of a genome complexity.WGS brought a required information to explain the occurred genomic events.
Trial registration number: NA

P-544 Comparison of aneuploidy rates between embryos obtained from fresh and frozen oocytes in the same patients: Analysis more than 1000 embryos in the UAE.

Y. Alhelou[1], S. El Shawarby[1], M. Fakih[1], A. Hellani[2], N.A. Mat Adenan[3], J. Ali[4]

[1]Fakih IVF Fertility Center, IVF Department, Abu Dhabi, United Arab Emirates ;
[2]Viafet genomics, PGD Lab, Dubai, United Arab Emirates ;
[3]University of Malaya, Obstetrics and Gynaecology, Kuala Lumpur, Malaysia ;
[4]University Of Malaya, Obstertrics and Gynaecology, Kuala Lumpur, Malaysia

Study question: Does oocyte vitrification affected the chromosomal content of the resulting embryos?
Summary answer: There is no significant aneuploidy rate between the embryos that resulted from fresh oocytes versus that resulted from frozen oocytes in the same patients.
What is known already: Oocyte vitrification is one of the routine practices in assisted reproduction. It gained more popularity after achieving high survival rates of more than 90% and pregnancy rates comparable to fresh oocytes. Therefore, there is a need to know if oocyte vitrification can affect embryo euploidy
Study design, size, duration: Retrospective data analysis was performed on 1050 embryos of 84 patients under age of 35 years between November 2017 and December 2019. Every patient was performed an oocyte vitrification cycle followed by a fresh ICSI cycle. On the same day of the fresh ICSI cycle, the vitrified eggs were warmed and ICSI was performed. The embryos resulting from both fresh and vitrified eggs had day 5 biopsy followed by PGS..
Participants/materials, setting, methods: Every patient was done an oocyte vitrification cycle followed by a fresh ICSI cycle. On the same day of the fresh ICSI cycle, the vitrified eggs were warmed and were done ICSI. The embryos resulted from both fresh and vitrified eggs were done day 5 biopsy followed by Preimplantaion Genetic Screening (PGS) using Next Generation Sequencing with High Resolution (NGS HR PGS). The structural and numerical chromosomal abnormalities were analysed and compared.
Main results and the role of chance: The biopsied embryo that resulted from fresh oocytes had an aneuploidy rate of 49.2% versus 53.8% aneuploidy rate for embryos resulting from frozen oocytes. The p value was 0.13 which means the result is statistically insignificant. In addition, the rate of monosomies, trisomies and complex abnormalities did not show any statistical significance.
Limitations, reasons for caution: The frozen/thawed oocytes were not recruited from the same fresh ICSI cycle. The retrospective nature of he study and the population of the study are all 35 years and younger
Wider implications of the findings: The study provides reassurance to young patients undergoing oocyte freezing for variance indications that the technology itself does not affect the rate of chromosomal abnormalities and aneuploidy rate.
As far as we know this is the first study in the UAE that investigate the aneuploidy in fresh and frozen oocytes.
Trial registration number: not applicable

P-545 Evaluation of Severe Male Infertility in the Light of New Genetic Variations

M. Gavaz[1], B. Umay Kara[1], F. Gokdagli Sagir[1], B. Yuksel[1], M. Cetinkaya[1], S. Kahraman[1]

[1]Istanbul Memorial Hospital, Assisted Reproductive Technologies and Reproductive Genetics Center, Istanbul, Turkey

Study question: Is it possible to classify the severe male infertility cases and predict their treatment outcome/prognosis according to causative genetic variation(s)?

Summary answer: The genetic variations identified in severe male infertility cases were correlated to the morphological sperm findings and the outcome with the genetic variations.

What is known already: The majority (30–60%) of infertile males do not receive a clear diagnosis; therefore, they are reported as idiopathic with a strong suspicion of genetic underpinnings. This is particularly evident in cases of infertility and repetitive ART failures with normal semen parameters. Genome-wide association studies in male infertility have affected several genomic regions, and all exome sequencing studies have identified coding variants associated with male infertility. WES has been successful in identifying new genes such as TEX15, MAGEB4, SUN5, SRA1 in all categories of etiologic spermatogenic dysfunction (quantitative, qualitative, hypothalamic-pituitary axis and ductal obstruction or dysfunction).

Study design, size, duration: We carried out a cohort study at Istanbul Memorial Hospital from December 2018 to December 2019. We included 25 patients with severe familial male infertility without identified etiology after standard genetic assessment (karyotype and Y chromosome microdeletion). We classified male infertility under 3 groups such as non-obstructive azoospermia (NOA) (n=7), oligoasthenoteratozoospermia (n=7), motility and morphological (n=11) defects. Successful treatment outcome was evaluated as being able to reach ongoing pregnancy.

Participants/materials, setting, methods: We obtained blood samples from all patients. Genomic DNA was extracted using the QIAampDNA Mini QIAcube Kit (Qiagen, France) according to the manufacturer's instructions. Whole exome sequencing was performed by NovaSeq 6000 Series Sequencer via Illumina technology. The coverage of target sequence sequencing is not less than 99%. Patients gave their informed consent to undergo genetic testing to uncover the etiology of male infertility.

Main results and the role of chance: Totally, 30 different previously identified genes that related with male infertility were detected. The most common variants were detected in NANOS1, AR, CCDC40 and RSPH1 genes. We detected five unique variants at NANOS1 gene in 5 patients. including a deletion of serine (p.S83del) residues that was identified as pathogenic. From five men with definitive sperm factors, two patients were identified heterozygous variations in CCDC40 gene: c.1318-14A>G which altered the protein sequence and resulted in the ultrastructural defects in the microtubule structure of cilia. In another patient, a variation in the CCDC40 gene that caused p.Asp284His at c.850G>C was identified as disease-causing. Different types of variants in the RSPH1 gene were detected in three patients with a history of parental consanguineous marriage. No positive pregnancy was obtained in NOA patients with variations in RSPH1, ZYMND15, SEPT12, DNAAF1, CCDC40 and NANOS1. Variation (c.849-20dup) in SPATA16 gene was found in 2 patients with morphological defect phenotype. Five of the sixteen patients with variants associated with primary ciliary dyskinesia had positive pregnancy and four had negative pregnancy, and three patients had been waiting for embryo transfer. Variants in the DNAAF5, RSPH1 and DNAI2 genes are the most common in patients resulting in negative pregnancies.

Limitations, reasons for caution: The main limitation of our study is the small number of patient. This study should be extended to include more patients from the same family who suffer from male infertility.

Wider implications of the findings: Our findings identified new variations responsible of the severe male infertility phenotype. This study provides an understanding of undefined variations involved in male spermatogenesis and progress in the diagnosis of infertility.

Trial registration number: -

P-546 Telomere length in oocyte- and sperm-derived metaphase chromosomes in human triploid zygotes

O. Efimova[1], A. Pendina[1], M. Krapivin[1], I. Mekina[2], E. Komarova[2], A. Tikhonov[1], O. Chiryaeva[1], Y. Sagurova[3], V. Baranov[1], A. Gzgzyan[2], I. Kogan[2]

[1]D.O. Ott Research Institute of Obstetrics- Gynecology and Reproductology, Genomic Medicine, St. Petersburg, Russia C.I.S. ;
[2]D.O. Ott Research Institute of Obstetrics- Gynecology and Reproductology, Assisted Reproductive Technologies, St. Petersburg, Russia C.I.S. ;
[3]St. Petersburg State University, Genetics and Biotechnology, St. Petersburg, Russia C.I.S.

Study question: Does telomere length in human triploid zygotes differ between oocyte- and sperm-derived sets of metaphase chromosomes and is it associated with maternal and paternal age?

Summary answer: In human triploid zygotes, sperm-derived telomeres are longer than oocyte-derived telomeres; telomere length does not depend on either maternal or paternal age.

What is known already: Telomeres represent complexes of short tandem DNA repeats and proteins that protect the ends of chromosomes. Telomere shortening caused by cell divisions, exogenous factors or genetic effects leads to chromosomal end fusion, degradation of chromosomes and cell death. Considering that telomere length (TL) in preimplantation embryo may be an important predictor of its developmental potential, the question about TL regulation in gametogenesis and embryogenesis is of high importance. In contrast to telomeres in sperm which become longer with age, oocyte telomeres are short. Whether these gamete-specific patterns are maintained after fertilization in zygote is unclear.

Study design, size, duration: A total of 23 triploid zygotes were obtained from 20 couples referred for in vitro fertilization during a ten month period. The maternal age ranged from 23 to 39 years (mean 32.04±0.8); the paternal age ranged from 23 to 45 years (mean 34.15±1.2). The study was approved by the Institutional Ethics Committee; the participating couples signed informed consent.

Participants/materials, setting, methods: For metaphase preparations, the zygotes were treated with colchicines, hypotonic solution and fixed on glass slides. Parental origin of chromosomes was identified immunocytochemically by weak DNA methylation and strong hydroxymethylation in the sperm-derived (paternal) chromosomes versus strong DNA methylation and weak hydroxymethylation in the oocyte-derived (maternal) chromosomes. Relative TL was assessed using quantitative fluorescence in situ hybridization (qFISH) (Telomere PNA FISH Kit/Cy3, Agilent) by dividing the telomeric fluorescence by the subtelomeric fluorescence measured in ImageJ1.49v.

Main results and the role of chance: Out of 23 triploid zygotes, four zygotes had additional maternal chromosome set and 19 zygotes had additional paternal chromosome set. According to the relative TL values, the zygotes were categorized into four types. In most zygotes (21 out of 23), the relative TL values were higher in the paternal chromosomes. In two zygotes, the relative TL values appeared to be higher in the maternal chromosomes. In two zygotes with additional paternal chromosome sets, only one out of two paternal sets had higher relative TL values compared to the maternal one. In two zygotes with additional maternal chromosome sets, only one out of two maternal sets had lower relative TL values compared to the paternal one. The Wilcoxon matched pairs signed rank test showed that the relative TL was significantly higher in the paternal chromosomes compared to the maternal ones ($p<0.0001$). A few zygotes demonstrating unusual patterns of the relative TL values seem to be the exception rather than the rule. This observation may suggest that some cases of developmental arrest in triploid embryos are due to the altered TL pattern. The relative TL neither in sperm-derived nor in oocyte-derived chromosomes correlated with paternal ($r=-0.058$; $p=0.736$) or maternal age ($r=0.155$; $p=0.44$), respectively.

Limitations, reasons for caution: As normal diploid zygotes were not available, the study was performed on triploid ones. The developmental potential of triploid human embryos is confirmed by their capacity for implantation and even a full-term development suggesting that basic developmental processes in triploid zygotes could be similar/identical to those in normal ones.

Wider implications of the findings: Our findings may suggest that after fertilization, TL in sperm-derived chromosomes is "reprogrammed". Being hypomethylated, paternal chromosomes are prone to recombination and, thus, to alternative lengthening of telomeres (ALT). ALT may be crucial for cleavage capacity and explains the absence of correlation of TL in sperm-derived chromosomes with paternal age.

Trial registration number: not applicable

P-547 Frequency of mitochondrial genetic variations in human cumulus cells and their association with mitochondrial function, embryo quality and BMI

K. Kumar[1], M. Venturas[2], D. Needleman[2], C. Racowsky[3], D. Wells[4]

[1]University Of Oxford, Nuffield Department of Women's & Reproductive Health, Oxford, United Kingdom ;
[2]Harvard University, MCB and SEAS, Cambridge- MA, U.S.A. ;
[3]Harvard Medical School, Brigham & Women's Hospital, Boston-MA, U.S.A. ;
[4]Juno Genetics Ltd, Juno Genetics Ltd, Oxford, United Kingdom

Study question: What is the frequency of mitochondrial genetic variations in human cumulus cells and are these associated with mitochondrial function, embryo quality or patient BMI?
Summary answer: Neither cumulus cell mtDNA quantity nor the presence of specific mtDNA genetic variants were associated with embryo quality. However, associations with patient BMI were detected.
What is known already: Cumulus cells fulfil a vital role in support of oocyte developmental competency. Cumulus cell function relies on adequate energy production, which in turn depends on the quantity and genetic competence of its mitochondria. Mitochondrial DNA mutations can be inherited or they can accumulate in somatic cells over time, potentially contributing to ageing. Such mutations may be homoplasmic (affecting all mtDNA in a cell) or they may display varying levels of heteroplasmy (affecting a proportion of the mtDNA). Currently, little is known concerning the frequency or associations between mitochondrial genetic variation in cumulus cells and oocyte health.
Study design, size, duration: Human cumulus cells, associated with 290 oocytes from 85 IVF patients, were analyzed. Patient age, body mass index (kg/m2), infertility diagnosis, fertilization status and embryo development were recorded with respect to each cumulus complex. Mitochondrial DNA quantity was measured using a validated quantitative PCR method. A subset of 119 samples had their entire mitochondrial genomes sequenced using a high-depth massively parallel sequencing approach. Metabolic functions were assessed non-invasively using fluorescence lifetime imaging microscopy (FLIM).
Participants/materials, setting, methods: Massive parallel sequencing not only permitted accurate detection of mutations, but also precise quantification of levels of mutations in cases of heteroplasmy. Sequence variants in the mtDNA were evaluated using Mitomaster and HmtVar to predict their potential impact. FLIM allowed measurement of the autofluorescence of NADH and FAD+, which are crucial for mitochondrial function. Multivariate generalized linear regression models were used to assess correlations among variables. P<0.05 was considered statistically significant.
Main results and the role of chance: Sequencing the entire mitochondrial genome in 119 cumulus cell samples revealed a total of 952 synonymous single nucleotide variants (SNVs) with respect to the reference genome (i.e. no amino acid change) and another 626 non-synonymous SNVs (altered amino acid sequence). Most SNVs were homoplasmic and therefore presumed to have been inherited, but 21 were heteroplasmic and potentially acquired by somatic mutation. Additionally, 104 homoplasmic variants detected in 63 samples were predicted to be pathogenic, with a likely impact on ATP production. A significant association was observed between BMI and the number of non-synonymous variants (p=0.037) and the quantity of mtDNA in cumulus cells (p<0.001). Significant difference was also observed for FLIM measurements of flavin adenine dinucleotide (FAD) long lifetime in cumulus cells that had pathogenic mtDNA mutations (p=0.046). Grouping samples according to patients' mitochondrial haplogroups revealed a significant association of nicotinamide adenine dinucleotide (NADH) long lifetime (p=0.025) and NADH short lifetime (p=0.012) with haplogroup. This suggests that mitochondrial metabolism varies between haplogroups. Moreover, the number of detected potentially pathogenic variants were also significantly different among mitochondrial haplogroups (p=0.005). No correlations were observed with respect to cumulus cell mtDNA quantity and patient age, infertility diagnosis, fertilization status or embryo quality.
Limitations, reasons for caution: Although, a large number of samples were analysed in this study, an expanded sample size is needed to determine whether subtle relationships do exist among between mtDNA mutation/copy number and patient/oocyte/embryo characteristics. We are continuing to process more samples.
Wider implications of the findings: No relationship was detected between cumulus cell mtDNA quantity or sequence variants (including pathogenic) and clinically relevant endpoints for corresponding oocytes. Nonetheless, some of the findings raise interesting biological questions, particularly regarding the interplay of metabolism and BMI.
Trial registration number: NOT APPLICABLE

P-548 A novel test for IVF miscarriage risk: Annexin A5 M2 haplotyping in IVF patients and preimplantation embryos

B.R. Rana[1], R. Zimmerman[1], D. Marin[1], J. Xu[1], E. Messick[1], L. Tellier[1], D. Egli[3], S. Fishel[2], N. Treff[1]

[1]Genomic Prediction Clinical Laboratory, Clinical Laboratory, North Brunswick, U.S.A. ;
[2]Care Fertility Group, Care Fertility Department of Research and Development, Nottingham, United Kingdom ;
[3]Columbia University, Department of Pediatrics, New York, U.S.A.

Study question: To develop a test for evaluating miscarriage risk in IVF patients and preimplantation embryos.
Summary answer: Development and validation of a novel M2 saliva test for screening patients with infertility, and the first method capable of M2 haplotyping in preimplantation embryos.
What is known already: Thrombophilia is linked to increased risk of miscarriage and other placental mediated pregnancy complications (PMPC). Expressed on the apical surface of the syncytiotrophoblast, the anticoagulant protein Annexin A5 (ANXA5), helps maintain placental vasculature. Genetic variation, known as the M2 haplotype, in the core promoter of the *ANXA5* gene reduces protein levels and increases the risk for adverse pregnancy outcomes. Infertile couples are 14% more likely to be carriers than the general population. Low dose heparin treatment is an effective strategy to combat the complications in carrier couples, but direct testing on preimplantation embryos may serve as a more effective alternative.
Study design, size, duration: Test performance was measured by comparing Sanger sequencing on parental blood DNA and quantitative real-time (q)PCR on saliva DNA, cell line 7-cell replicates, cell line 7-cell samples and corresponding purified DNA, trophectoderm biopsies and DNA isolated from the corresponding embryonic stem cell line, Mendelian inheritance expectations in embryos, embryo sanger sequencing, and SNP microarray-based linkage analyses. Embryo qPCR allele dropout rates, M2 frequencies observed in an IVF patient population, and inheritance patterns in IVF-derived embryos.
Participants/materials, setting, methods: Initial assay development was performed on 6 IVF patient samples. Additional data was obtained from 14 cases of PGT where parental and embryo biopsy DNA (n=107) were available for research and 55 patients ordering clinical M2 testing. M2 testing was performed at Genomic Prediction Clinical Laboratory under CLIA certification and CAP accreditation.
Main results and the role of chance: Concordance rates between sanger sequencing and qPCR was 93% (13/14) on parental blood DNA and 100% (6/6) on saliva DNA. Concordance rates between all replicates of the cell line 7-cell samples were 100% (24/24). Concordance rates between 7-cell aliquots and corresponding bulk DNA was 100% (20/20). The concordance rate between trophectoderm biopsies and DNA isolated from the corresponding embryonic stem cell line was 100% (1/1). Concordance between qPCR and Sanger sequencing on trophectoderm biopsies was 100% (2/2). Trophectoderm biopsy qPCR results were 97% (104/107) concordant with expectations from Mendelian inheritance rules and 100% (107/107) when including SNP array based linkage analyses. There was an M2 frequency rate of 18% in patients ordering clinical testing. When one parent was a carrier, half (33/66) of the embryos were M2 carriers. In families where both parents were carriers, 76% (16/21) of embryos were M2 carriers.
Limitations, reasons for caution: Embryo biopsy qPCR displayed a 7% allele drop out rate and required concurrent SNP array based linkage analysis to obtain accurate genotypes.
Wider implications of the findings: We have developed and validated a saliva DNA-based M2 haplotyping test for infertile patients, and a new ability to perform "PGT-M2 haplotyping" for carrier couples undergoing IVF. Future work will include prospective analysis of clinical outcomes following embryo selection and non-selection with PGT for M2 carrier status.
Trial registration number: not applicable

POSTER VIEWING SESSION
REPRODUCTIVE ENDOCRINOLOGY

P-549 None of The Parameters of Bologna and Poseidon Criteria Has a Significant Impact on Pregnancy Rates

B. Devranoglu[1], H. Bozdag[2], E. Özkaya[1], Ç. Yayla Abide[1], E. Çöğendez[1], E. Tozkır[1], E. Darici[1]

[1]University of Health Science- Zeynep Kamil Maternity and Childeren's Diseases Health Training and Research Hospital, Department of Obstetrics and Gynecology, istanbul, Turkey ;
[2]Health Sciences University Sultan Abdülhamid Han Training and Research Hospital, Department of Obstetrics and Gynecology-, istanbul, Turkey

Study question: What are predictive values of the individual parameters of Bologna and Poseidon criteria for clinical pregnancy and cycle cancellation in women with poor ovarian reserve or previous poor response?
Summary answer: These criteria, Bologna or Poseidon, did not have any benefit to ameliorate management to obtain a better outcome.
What is known already: Even several authors have attempted to define the diagnosis of poor ovarian response (POR), this terminology has not been clarified among international publications. According to a published systematic review of 47 randomized controlled trials, there were 41 different POR definitions. To outline the POR definition in a standardized manner, Ferraretti et al. have proposed new definitive criteria known as "Bologna Criteria.". A New strategy, POSEIDON, has been introduced to predict the prognosis of patients undergoing ovarian stimulation for IVF.
Study design, size, duration: Seven hundred twenty-two cases were retrospectively screened from the database and reclassified based on both Bologna and Poseidon criteria separately, and assisted reproductive technology (ART) outcomes were compared for each corresponding group. The diminished ovarian reserve was defined according to basal follicle-stimulating hormone (FSH) value >10 IU/L, antral follicle count <6, or anti-Müllerian hormone <1.1 ng/mL and/or a previous poor ovarian response. The presence of one of these criteria made us diagnose poor ovarian reserve.
Participants/materials, setting, methods: Poor ovarian reserve was determined in patients who fulfilled the above-mentioned criteria. Individualized gonadotropin dose and protocol were selected based on our institutional conventional ovarian hyperstimulation protocols defined for cases with poor ovarian reserve. All these cases were retrospectively screened from the database and reclassified based on both Bologna and Poseidon criteria separately, and ART outcomes were compared for each corresponding group.
Main results and the role of chance: The highest pregnancy rate was observed in Poseidon group 1 (10.1 % vs. 4.8 % in Poseidon group 2, 4.6 % in Poseidon group 3, 4.3 % in Poseidon group 4) however the difference between groups did not reach statistical significance ($p > 0.05$). No pregnancy was observed in Bologna groups which included cases fulfilled the criteria age and ovarian reserve or age and oocyte number, on the other hand, Bologna group with oocyte number and ovarian reserve criteria had 4.7 % pregnancy rate, and pregnancy rate was 3.7 % in group of women who fulfilled all criteria of Bologna classification. Pregnancy rates were not found to be statistically significant ($p > 0.05$).
Limitations, reasons for caution: This was a retrospective study and no data available in terms ongoing and live birth rates.
Wider implications of the findings: Our data showed that Bologna or Poseidon did not have any benefit to change management to obtain better outcomes but may be utilized to provide better and precise information and counseling for the patients with poor ART outcomes before starting the treatment cycle.
Trial registration number: not applicable

P-550 Adenosine deaminase activity in the follicular fluid of infertile women with diminished ovarian reserve can act as a predictor of ovarian reserve

G. Keskin[1], B. Bacanakgil[1], H.H. Kalem[2], A. Köse[2], A. Atıcı[1], S. Yalcınkaya[1], M. Ayyildiz[3]

[1]Health Science University Istanbul Research and Education Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey ;
[2]Health Science University Istanbul Research and Education Hospital, Department of Embriyology, Istanbul, Turkey ;
[3]Health Science University Istanbul Research and Education Hospital, Department of Biochemistry, Istanbul, Turkey

Study question: Does the activity of total Adenosine deaminase (ADAT) in the follicular fluid have a relation with ovarian reserve and clinical parameters linked to IVF outcomes?
Summary answer: Follicular fluid(FF) ADAT activity in DOR group was higher and had a negative correlation with BMI,a positive correlation with FSH and no relation with IVFoutcomes.
What is known already: In the human reproductive tract,the function of the enzymes that affect adenosine metabolism,especially ADA,have been investigated.The activity of ADA was evaluated in the male reproductive system. Inhibition of ADA activity increases adenosine levels and it resulted an improvement of sperm fertilisation.In addition, it is suggested that ADA receptors might have a role in capacitation and acrosome reaction.Alteration in ADA activity seemed to be related with male infertility.For the female reproductive system,that women with elevated ADA activity have the highest risk for recurrent spontaneous abortion.To the best of our knowledge,ADA activity has not yet been systematically addressed in the female infertility.
Study design, size, duration: This study is a retrospective cohort study. The data of all individuals, who underwent their IVF/ICSI at IVF Unit of Istanbul Research and Education Hospital from May 1st 2019 to July 31st 2019 were retrospectively reviewed. Total of 106 women were eligible and enrolled in this study.
Participants/materials, setting, methods: The women were between 23–45 years old, had an infertility etiology of diminished ovarian reserve(DOR),tubal factor(TF),male factor(MF) or unexplained infertility. Patients were excluded if they had two or more type of infertility etiologies, diseases of the immune system, hematologic malignancies, ovarian tumors, endocrine disorders or smoking. Blood samples were drawn before oocyte retrieval. FF from the first aspirated follicle was used for ADAT measurement.Its relation with ovarian reserve markers and IVF outcomes were determined.
Main results and the role of chance: A total of 106 infertile women were enrolled in the study. Thirty-three of the women had DOR, 22 had TF, 21 had unexplained infertility and 30 had MF infertility.

AMH level of DOR group was significantly lower than that of the other groups ($p<0.01$). FSH level of DOR group was significantly higher than that of the other groups ($p<0.05$). Mean level of serum estradiol on the day of hCG administration ($p<0.01$), total number of oocytes retrieved ($p<0.01$) and MII oocyte count ($p<0.01$) of DOR group were significantly lower than that of the other groups. There was no statistically significant difference among the other groups ($p>0.05$).

Plasma lymphocyte, monocyte and eosinophil counts were not statistically different between groups ($p>0.05$).

Activity of plasma ADAT was significantly higher than activity of FF ADAT in all of the infertile groups ($p<0.01$). Activity of FF ADAT was higher in DOR group than the others ($p<0.01$).There was no statistically significant difference among the other groups ($p> 0.05$).

The relation of ADAT activity with BMI showed a negative correlation in DOR group (r:-0.507). Additionally, ADAT activity of DOR group and FSH levels, showed a positive correlation (r:0.352).
Limitations, reasons for caution: Study population was small and ADA enzyme activity could be calculated as ADA1 and ADA2 rather than ADAT.
Wider implications of the findings: Increased ADAT activity can lead to reduced adenosine levels, which might be resulted in disturbed fertility process. The activity of FF ADAT activity might be important for fertility work-up. Further studies are needed.
Trial registration number: IRB: IEAH7/2019

P-551 Predictive factors influencing pregnancy rate in frozen embryo transfer.

N. Hayashi[1], N. Enatsu[1], T. Iwasaki[1], J. Otsuki[2], Y. Matsumoto[1], S. Kokeguchi[1], M. Shiotani[1]

[1]Hanabusa Women's clinic, medical office, Kobe, Japan ;
[2]Okayama University Assisted Reproduction Technology Center, medical office, Okayama, Japan

Study question: What factors influence pregnancy rate (PR) in frozen embryo transfer (FET)?
Summary answer: Endometrial thickness and the position of transferred air bubbles influenced clinical pregnancy in FET cycles.
What is known already: There have been many reports evaluating single factors which affect embryo transfer (ET) outcomes including embryo quality,

female age, endometrial thickness and the technique of the transfer procedure. Most studies have confirmed that the PR is better in women with a thicker endometrium than those with a thinner endometrium. Another significant variable was the position of the transferred air bubble, which is often regarded as an indicator for the position of the transferred embryo. However, precisely which factors and the degree to which they each affect ET outcomes is still unknown.

Study design, size, duration: This study was a retrospective study, and included 938 FET cycles involving single frozen-thawed good quality blastocyst (Gardner grade ≥ 3BB) between August 2017 and January 2018 in a single fertility clinic.

Participants/materials, setting, methods: FET was performed with either a hormone-replacement cycle or natural-cycle protocol. The primary outcome of this study was clinical pregnancy defined as the detection of gestational sac in the uterus by using transvaginal ultrasound at 5 weeks gestational age. The significance of several parameters including endometrial thickness, position of the transferred air bubble, self-evaluation score by physicians, and the uterus direction at ET as predictors of clinical pregnancy were evaluated using univariate and multivariate analysis.

Main results and the role of chance: Among 938 ET cycles, 462 (49.3%) were considered clinically pregnant. Among the variables, endometrial thickness was positively associated with clinical pregnancy in a linear trend. The clinically acceptable threshold was calculated as 10.0mm. The position of the transferred air bubble and the clinical pregnancy rate showed a curvilinear relationship indicating that the clinical PR increased as the air bubble position got closer to 6 mm apart from uterine fundus, remained steady until 10 mm, and began decreasing after 10 mm. The clinical PR was significantly higher in cases with a self-evaluation rating of A, compared with ratings of B or C (50.2% vs 42.1%, p<.05). Comparing the clinical PR based on uterus direction, there was no difference between the anteflexion, retroflexion, and straight groups (59.5%, 47.6%, and 42.9% respectively, p = .45). Univariate analysis of predictive parameters identified endometrial thickness, self-evaluation score by physicians, and position of air bubbles as significant predictors of clinical pregnancy, of which endometrial thickness and position of air bubbles appeared to be independently related to clinical pregnancy (odds ratio; 1.56 and 1.34, 95% confidence interval; 1.20 – 2.03 and 1.02 –1.77 respectively, p<.05).

Limitations, reasons for caution: Patients with slight complication such as leiomyoma or hydrosalpinx without surgical indication and adenomyosis were included in this study. Therefore, we could not completely exclude the influence of these factors Additionally, this study was a non-randomized, retrospective study, and a larger prospective study would be needed to optimize ET strategy.

Wider implications of the findings: We recommend that physicians should focus on maintaining their patients' endometrial at the greatest possible thickness during the ET cycle. Moreover, the transferred air bubbles, that means a position of the transferred embryo, should be placed between 6 to 10 mm from the fundus during ET procedure.

Trial registration number: not applicable

P-552 Analysis of Nonylphenols (NPs), Mirex and selected endocrine disrupting chemicals (EDCs) in the follicular fluid (FF) of women undergoing intracytoplasmic sperm injection (ICSI)

T. Trapphoff[1], A. Bullach[2], S. Zühlke[2], M. Spiteller[2], S. Dieterle[1]

[1]Kinderwunschzentrum Dortmund, Dortmund Fertility Centre, Dortmund, Germany ; [2]Technische Universität Dortmund, Analytische Chemie und Umweltchemie INFU, Dortmund, Germany

Study question: Are selected EDCs including Mirex and NPs present in the FF of women undergoing ICSI, and are they associated with clinical and socioeconomic parameters?

Summary answer: NP, Mirex and selected EDCs can be quantified in FF and may lead to a reduced maturation and fertilization rate in patients undergoing ART.

What is known already: EDCs are exogenous substances known to interfere with the mammalian hormone system. EDCs mostly origin from synthetic chemicals used in industry, pharmacy or agriculture. Exposure to EDCs has been shown to affect female reproduction. It is known that different EDCs alter the embryonic development, spindle formation, chromosomal alignment or gene expression of oocytes and embryos. Besides well studied EDCs like Polychlorinatedbiphenyls (PCB), Polybrominateddiphenylethers (PBDE) or Dichlorodiphenyltrichloroethane (DDT)/Dichlorodiphenyldichloroethylene (DDE), less is known about two extensively utilized EDCs: Mirex and the technical mixture of nonylphenols.

Study design, size, duration: Consecutive patients undergoing ICSI due to male subfertility were included. Patients with endometriosis or polycystic ovaries were excluded. Human FF was collected at the Fertility Center Dortmund between 2016 and 2017 (*n*=210). Only FF from the first two punctured follicles was collected. An additional multiple-choice questionnaire was provided to identify potential sources of EDC, because the social environment, consumers behaviour and food habits may play an essential role regarding EDC uptake.

Participants/materials, setting, methods: The concentration of Mirex, five different NP isomers, DDT/DDE, six PCB and four PBDE congeners in the FF was analysed using gas-chromatography coupled with mass-spectrometry using selected ion monitoring. The samples were extracted and derivatized before quantification of selected EDC. Standard solutions of pre-named concentrations and solvent blanks were injected with every measurement. To identify a potential association between EDC-exposure, -uptake, clinical parameters and socioeconomic factors, a statistical evaluation was performed.

Main results and the role of chance: All investigated EDCs were present in every FF sample showing a broad distribution of concentrations between individual samples. NP was present in the FF at 10.4±11.1ng/g FF [mean±sd], Mirex at 1.1±0.9ng/g FF, PCBs at 0.1±0.1ng/g FF, PBDEs at 0.04±0.02ng/g FF and DDT/DDE at approximately 0.7±0.8ng/g FF. There was no significant correlation between EDC concentration and the number of retrieved oocytes. With regard to clinical parameters, an increased concentration of NP, Mirex, DDT/DDE, PCB153, PBDE99, 100 and BB153 in FF samples correlated negatively with the maturation rate (p<0.05). Nearly all examined EDCs including NP isomers and Mirex lead to a relative decrease in the number of 2PN and hence a significantly reduced fertilization rate (p<0.05). In contrast to the maturation and fertilization rate, no significant influence of EDC concentration on clinical pregnancy rate could be observed.

Regarding socioeconomic factors, no impact was observed regarding patients' residential area, diet, source of food products, nicotine or caffeine consume, stays abroad as well as professional life. However, a significant correlation between EDC concentration and source of supply of textiles (fashion discounters vs. retail shop vs. fashion boutiques) was found (p<0.05), in particular for NP.

Limitations, reasons for caution: Limitations of this study include difficulties in extrapolating the findings to the general population, because no data of women not undergoing ICSI are available. Data regarding the exact molecular mechanism of NP and Mirex respectively, are limited.

Wider implications of the findings: EDC uptake and exposure to NP, Mirex, PCB, PBDE or DDT/DDE may adversely affect female reproductive outcome. Higher EDC levels in the FF correlate negatively with the maturation and fertilization. Consumers´ behaviour play an essential role regarding the individual EDC uptake.

Trial registration number: NCT01385605

P-553 Aging attenuates ovarian circadian rhythm

G.L. Ding[1], Z.R. Jiang[1], K.X. Zou[1], H.F. Huang[1]

[1]The International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Study question: The objective of our study was to investigate the effect of aging on ovarian circadian rhythm.

Summary answer: Subfertility in older women is partially due to ovarian circadian dysrhythmia as a result of aging.

What is known already: The circadian clock is also known as the physiological clock. In mammals, the circadian clock system is composed of coordinated and synchronized cell and tissue clocks, including the central clock located in the suprachiasmatic nucleus (SCN) of the basal hypothalamus and the peripheral clock in the various tissues of the body.The ovarian circadian clock is a peripheral circadian clock that is regulated by neuroendocrine signals from the SCN, playing an important role in the physiological process of the normal reproductive system, such as ovulation and steroid release. However, the effect of aging on the ovarian clock has hardly been explored.

Study design, size, duration: Human granulosa cells were obtained by follicular aspiration from women undergoing in vitro fertilization (IVF), which were divided into two groups, 34 cases of young group (under 40 years old) and 18

cases of old group (over 40 years old). Female C57BL/6 mice of 12-week-old were determined as young group and 8-month-old as old group. Three to five mice per group were sacrificed every fourth hour to collect liver and ovary tissues.

Participants/materials, setting, methods: The ovarian luteinized granulosa cells from young and old patients were isolated. Anti-Müllerian hormone (AMH) was examined. For mice, the stage of the estrous cycle was determined by vaginal smears. During proestrus, three to five mice per group were sacrificed every fourth hour (ZT 0, ZT 4, ZT 8, ZT 12, ZT 16, ZT 20) to collect liver and ovarian tissues. Real-time quantitative PCR was used to detect the expression of clock genes.

Main results and the role of chance: All the examined circadian clock genes (Clock, Bmal1, Per1, Per2, Cry1 and Rev-erbα) in human granulosa cells showed a downward trend in expression with aging. And, the mRNA expression levels of the circadian clock genes were negatively correlated with age ($P<0.05$). Older patients (≥ 40 years of age) had significantly reduced serum anti-Müllerian hormone (AMH) levels. Except for Rev-erbα, all the other examined circadian clock genes were positively correlated with the level of AMH ($P<0.05$). The circadian rhythm in the ovaries of older mice was changed significantly, although the circadian rhythm in the livers of older mice was basically consistent with that of young mice. There was no difference between the two groups in the expression of the differentiation marker LHCGR and the expression of the internal reference β-actin, suggesting that the decreased expression of clock genes was indeed related to age but was not related to a reduction in the extent of granulosa cell differentiation.

Limitations, reasons for caution: Our findings from human granulosa cells suggested a difference in the expression of circadian clock genes at certain time points. But the effect of aging on the rhythm of the ovarian clock could not be verified in human samples. So we also carried out mouse experiments in our study.

Wider implications of the findings: In our study, we found that age is closely related to ovarian circadian clock. Subfertility in older women is partially due to ovarian circadian dysrhythmia as a result of aging. More studies will be needed to explain mechanistically how aging affects the circadian clock of the ovary.

Trial registration number: not applicable

P-554 Embryo transfer strategy for patients with normal P level on hCG day: a large sample retrospective cohort study

S. Yang[1], L. Rong[1], Z. Xiumei[1], W. Lina[1], W. Haiyan[1], M. Caihong[1], L. Ping[1], Q. Jie[1]

[1]Peking University Third Hospital, Reproductive Medical Center, Beijing, China

Study question: The present study was aimed to investigate the transfer strategy for patients with different level of "normal" P level on hCG day.

Summary answer: The clinical outcomes of fresh embryo transfer were better with lower P level on hCG day.

What is known already: The "normal" P level might also affect the clinical outcomes of IVF/ICSI. More and more clinicians consider the "frozen-all" strategy safe and effective, but is this strategy proper for everyone and is there more individualized choice for patients?

Study design, size, duration: It is a single-center retrospective cohort study, and the study period was patients accepted oocyte pick-up between 1st Jan. 2011 and 31th Dec. 2016. During the study period, 20,658 cycles were full-filled the inclusive and exclusive criteria, including 4915 cycles of frozen-all patients had accepted frozen-thawed embryo transfer by the end of Jun. 2019 (first frozen-thawed cycles after frozen-all in fresh cycles). There were 15,473 fresh cycles and 4,915 frozen-thawed cycles were analyzed respectively.

Participants/materials, setting, methods: The cycles were divided into two groups depending on the embryo transfer strategy, fresh embryo transfer or frozen-all. And both groups were further divided into four subgroups depending on Quartile of P level and embryo stage (D3/D5).

Main results and the role of chance: The clinical outcomes of fresh embryo transfer refer to clinical pregnancy rate (CPR), implantation rate (IR), and live birth rate (LBR) were decreased with increasing P level, especially when P>3.56nmol/l, which were lower than those accepted frozen-thawed embryo transfer ($p<0.05$). The patients accepted D3 embryo transfer and P≤3.56nmol/L, the fresh embryo transfer outcomes were better than frozen-thawed cycles, including higher CPR, IR, LBR, and lower miscarriage rate ($p<0.05$). The clinical outcomes were comparable in blastocyst embryo transfer cycles, between different P level with fresh or frozen-thawed embryo transfer. The clinical outcomes of frozen-thawed cycles were comparable between patients with different P level on hCG day. For patients with P ≤3.56nmol/L, we suggested fresh embryo transfer. And for those p>3.56 nmol/L, we suggested blastocyst embryo transfer with fresh embryo or frozen-thawed embryo transfer.

Limitations, reasons for caution: The present study was a single center retrospective study, the cut-off level of progesterone only suitable for our center.

Wider implications of the findings: The present study suggest individualized embryo transfer strategy for patients with different progesterone levels, and pointed out that there should not be "one for all" strategy in IVF/ICSI cycles.''

Trial registration number: NOT APPLICABLE

P-555 Melatonin – a new fertility wonder drug? A systematic review on the impact of melatonin on fertility

M. Mejlhede[1], U.B. Knudsen[2]

[1]Aarhus University, Health, Aarhus, Denmark ;
[2]Aarhus University, Clinical Institute of Medicine, Aarhus, Denmark

Study question: How does melatonin effect assisted reproductive technologies (ART)-outcomes?

Summary answer: Most of the included studies showed a significant improvement in one or more ART-outcome but not in clinical pregnancy rate or live birth rate.

What is known already: ART are associated with significant levels of oxidative stress, which can have a negative impact on the pregnancy rates by modifying the quality of oocytes, embryos and sperm. The anti-oxidant melatonin has been investigated as a supplement in *in vitro* fertilization embryo-transfer (IVF-ET) protocols to see if it can improve the ART-outcomes.

Study design, size, duration: A systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Systematic data-search of PubMed, Embase and Cochrane Central Register of Studies from the earliest available online year of indexing up to April 2019 was conducted. Fifteen trials were included. The total number of patients was 1.781. One retrospective cohort study reported the results in embryo transfer cycles and thereby contributing with 13.372 unique embryo transfer cycles.

Participants/materials, setting, methods: The population consisted of infertile women/men undergoing IVF-ET including intracytoplasmic sperm injection (ICSI).

The intervention was melatonin in vivo/vitro.

The primary outcome was clinical pregnancy rate (CPR).

The secondary outcomes were live birth rate (LBR), sperm quality-parameters, fertilization rate, oocytequantity/quality, quality of embryos and implantation rate.

Methodological quality was assessed for each study using the CHECKLIST from the European Society of Human Reproduction and Embryology (ESHRE).

Main results and the role of chance: No significant changes in CPR in relation to use of melatonin were demonstrated. The majority of studies did not show effect on LBR. One study showed a significant reduction in LBR. Three studies showed a significant improvement of fertilization rate and one study showed a significantly higher implantation rate. Eight studies demonstrated a significant rise in embryo quality. Oocyte quality and/or quantity were showed to be significant improved by seven studies. The two studies, who investigated sperm quality parameters, both demonstrated a significant improvement in the measured parameters. Ten studies had poor methodological quality.

Limitations, reasons for caution: The included studies were small and the methodological quality of the studies low or very low. Therefore, there is insufficient evidence to make any conclusion on whether melatonin could influence the result of ART.

Wider implications of the findings: A systematic review and meta-analysis from 2013 demonstrated a pooled risk ratio of 1.21 for CPR in favour of melatonin. A Cochrane Systematic Review investigated the effects of use of antioxidants including melatonin on female subfertility. The CPR improved (not statistically significant) in the group receiving melatonin compared to control-group.

Trial registration number: not applicable

P-556 The transfer of an extra low quality embryo: Is it ever worth it?

D. Braga[1,2], A. Setti[1,2], R. Ferreira[3], A. Iaconelli Jr.[4], E. Borges Jr.[2,4]

[1]Fertility Medical Group, Scientific research, Sao Paulo, Brazil ;
[2]Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Assistida, Scientific research, Sao Paulo, Brazil ;
[3]Fertility Medical Group, IVF laboratory, Sao Paulo, Brazil ;
[4]Fertility Medical Group, Clinical department, Sao Paulo, Brazil

Study question: Is it ever worth it to add an extra low-quality embryo to a high-quality embryo transfer in IVF cycles?
Summary answer: The transfer of an extra low-quality embryo is worth it for patients >37 years-old. For younger women, this dramatically increases the risk of multiple pregnancies.
What is known already: Although morphological evaluation has been the main strategy applied in order to select embryos for transfer, it has been shown that even aneuploid embryos are able to reach high morphological scores and vice versa. In an effort to promote singleton gestation and reduce the number of multiple pregnancies, guidelines for the limits on the number of embryos to be transferred in IVF cycles have been published. Recommendations strict the number of embryos transferred considering patients age and treatment prognosis. Information concerning the developmental stage is also provided, however, less is known about the quality of transferred embryos.
Study design, size, duration: Data analyzed in this historical cohort study were obtained via chart review of 1249 patients undergoing intracytoplasmic sperm injection (ICSI) cycles between 2016 and 2018. For all cycles one or two embryos were transferred on day five. The embryo morphology was assessed 16–18h post-ICSI and on the mornings of days two, three, and five of embryo development and all evaluated parameters were used for the classification of embryos into top- or low-quality embryo.
Participants/materials, setting, methods: This study was performed in a private university–affiliated IVF center. Cycles were split into groups depending on the number and quality of transferred embryos. Group Top-Quality (n=871), in which a single top-quality embryo was transferred, Group Mixed-Quality (n=378), in which one top-quality and one low-quality embryo were transferred. The impact of the transfer of extra low-quality embryos on singleton and multiple pregnancies rates, in different female age ranges was investigated by general mixed models.
Main results and the role of chance: The pregnancy rate was significantly increased when the double embryo transfer, including one top-quality and one low-quality embryo, was compared with the single top-quality embryo transfer, regardless of the age: 33.5% vs 44.6%, p=0.003, for patients <35 years-old, 27.9% vs 36.9%, p<0.027, for patients between 35 and 37 year-old, and 25.4% vs 24.1%, p=0.041 for patients >37 years-old, for Top-Quality and Mixed-quality groups respectively. The transfer of an extra low-quality embryo also increased the multiple pregnancy rates in younger patients: 0.0% vs 41.3%, p<0.001, for patients <35 years-old and 27.9% vs 36.9%, p=<0.001 for patients between 35 and 37 year-old, for Top-Quality and Mixed-quality groups respectively. For patients >37 years-old, no differences in the multiple pregnancy rate was noted when one (high-quality) or two (one high- and on low-quality) embryos were transferred: 15.3% vs 21.1%, p=0.694, for Top-Quality and Mixed-Quality groups respectively. The increment of one low-quality embryo increased the odds of multiple pregnancies in 37% for patients <35 years-old (OR: 0.631, CI: 0.487 - 0.817, p<0.001) and in 44% in patients between 35 and 37 year old (OR: 0.563, CI: 0.340 – 0.932, p=0.026). The miscarriage rates did not differ among the groups, for any age.
Limitations, reasons for caution: Retrospective nature of this study and the small sample size may be a reason for caution.
Wider implications of the findings: The addition of an extra low-quality embryo may increase the pregnancy rate and be valuable for older patients, in which the pregnancy chance is lower. However, for younger patients, this results in increased multiple pregnancy chance, raising the question on whether taking on this level of risk is worth it.
Trial registration number: Not applicable

P-557 The contribution of the rescue in vitro maturation to ICSI cycles

R.C. Ferreira[1], D. Braga[2,3], A. Setti[2,3], A. Iaconelli Jr.[4], E. Borges Jr.[3,4]

[1]Fertility Medical Group, IVF Laboratory, São Paulo, Brazil ;
[2]Fertility Medical Group, Scientific research, São Paulo, Brazil ;
[3]Instituto Sapientiae - Centro de Estudos e Pesquisa em Reprodução Assistida, Scientific research, São Paulo, Brazil ;
[4]Fertility Medical Group, Clinical department, São Paulo, Brazil

Study question: Is it ever worth it to add an extra embryo derived from an immature oocyte, when an embryo derived from metaphase-II (MII) oocyte is transferred?
Summary answer: The transfer of an extra embryo, derived from rescue in vitro maturation (rIVM) is not worth it.
What is known already: In vitro maturation (IVM) has been reported mostly in women with polycystic ovary syndrome, where, oocytes are on purpose recovery at immature stages in natural cycles or after low-dose gonadotropin stimulation. Success rates after IVM appear inferior to in vivo-matured oocytes. Unlike in scheduled IVM, in stimulated cycles, pharmacologic doses of gonadotrophins induce the growth of follicles, which, under natural conditions, would become atretic. Ovarian stimulation leads to the retrieval of oocytes at different nuclear stages and some may spontaneously mature in vitro. Whether rIVM of oocytes retrieved from stimulated cycles may improve the ICSI outcomes is to be elucidated.
Study design, size, duration: Data analyzed in this historical cohort study were obtained via chart review of 2021 patients undergoing controlled ovarian stimulation for ICSI cycles, between 2016 and 2018. After ovum-pickup oocytes were assessed for nuclear status. Mature oocytes that had released the first polar body were used for ICSI. Immature oocytes, at prophase I (PI) and metaphase I (MI) stages, were cultured. Those that spontaneously matured in vitro were eventually injected and transferred.
Participants/materials, setting, methods: Cycles, performed in a private university–affiliated IVF-center, were split into groups depending on the origin of transferred embryos: Group-Single-MII (n=454, one embryo, derived from an MII-oocyte was transferred), Group-Double-MII (n=1483, two embryos, derived from MII-oocytes were transferred, Group-Double-Mixed-MI (n=72, one embryo derived from an MII-oocyte and one from a MI-oocyte were transferred, and Group-Double-Mixed-PI (n=12, one embryo derived from an MII-oocyte and one from a PI-oocyte were transferred). Outcomes were compared among the groups.
Main results and the role of chance: Overall 21,220 oocytes were retrieved, in which 15,200 (71.6%) were in the MII-stage, 1830 (8.6%) were in the MI-stage, and 4,190 (19.7%) were in the PI stage. When adjusted for the age and endometrial thickness, the pregnancy rate was increased when two MII-derived embryos were transfer in comparison with the transfer of a single MII-derived embryo, however, no significant differences were noted when a single MII-derived embryo transfer or a double embryo transfer, including one MII-derived embryo and one MI- or one P1-derived embryo, was performed (18.0%, 39.4%, 33.3% and 15.3.0%, p<0.001, for Group-Single-MII, Group-Double-MII, Group-Double-Mixed-MI and Group-Double-Mixed-PI, respectively). The same results were observed for the implantation rate: the Group-Double-MII, presented the higher implantation, followed by the Group-Single-MII and Group-Double-Mixed-MI, while the Group-Double-Mixed-PI, presented the lowest rate (17.7%, 29.4%, 20.8% and 11.8%, p<0.001, for Group-Single-MII, Group-Double-MII, Group-Double-Mixed-MI and Group-Double-Mixed-PI, respectively). The miscarriage rate did not differ among the groups (9.9%, 9.3%, 9.1% and 25.0%, p=0.911, for Group-Single-MII, Group-Double-MII, Group-Double-Mixed-MI and Group-Double-Mixed-PI, respectively).
Limitations, reasons for caution: The embryo quality may be a source of bias and a reason for caution.
Wider implications of the findings: These findings raise the question about the contribution of rIVM in ICSI cycles. Although previous studies demonstrated that rIVM may increase the number of embryos available for transfer, the quality of these embryos must be carefully evaluated, since its implantation potential seems to be limited, especially for PI-derived embryos.
Trial registration number: Not Applicable

P-558 Serum sex hormone–binding globulin (SHBG) levels during controlled ovarian hyperstimulation as a predictor of ovarian response in women without polycystic ovary syndrome

J. Zhai[1], S. Li[1], Y. Zhu[1], Z.J. Chen[1], Y. Du[1]

[1]Ren Ji Hospital- School of Medicine- Shanghai Jiao Tong University, Center for Reproductive Medicine, Shanghai, China

Study question: Is the serum SHBG level during controlled ovarian hyperstimulation (COH) associated with ovarian response? What's the difference between polycystic ovary syndrome (PCOS) and non-PCOS patients?
Summary answer: Serum SHBG levels, especially SHBG on hCG-day, could be recognized as a predictor of ovarian response during COH in non-PCOS, but not in PCOS participants.
What is known already: Previous study reports the positive correlation of serum SHBG levels with the total number of follicles in infertile women with tubal and/or male-factor who undergo IVF and Recent literature has also stressed the importance of SHBG measurement in the diagnosis and management of PCOS. However, the correlation between serum SHBG levels and ovarian response in COH cycles and the difference between PCOS and non-PCOS patients remain unclear and need to be investigated.
Study design, size, duration: This is a prospective study. A total of 120 participants undergoing the first IVF cycle were recruited to our study. The serum SHBG levels were detected to analyze the role of SHBG in the prediction of ovarian response in both PCOS and non-PCOS patients. The duration of this study is from August 1st, 2018 to October 1st, 2019.
Participants/materials, setting, methods: A total of 120 participants (60 non-PCOS and 60 PCOS participants) receiving GnRH-antagonist protocol were recruited to our study. The serum samples were collected every 2 days from each participant and the concentrations of serum SHBG and sex hormones were tested to investigate the relationship between serum SHBG levels and ovarian response during COH. Additionally, the number of follicles and the endometrium thickness were monitored using B-ultrasound.
Main results and the role of chance: Serum SHBG concentrations decreased along the COH cycle of GnRH antagonist protocol both in PCOS and non-PCOS participants. Serum SHBG levels on human chorionic gonadotropin (hCG)-day were positively correlated to numbers of retrieved oocyte and embryo in all participants. Furthermore, both basal and hCG-day SHBG levels of high responders (>15 retrieved oocytes) significantly increased compared with those of normoresponders (4-15 retrieved oocytes) in the analysis of all participant (n=120). However, when we analyzed separately, the positive correlation between serum SHBG levels and ovarian response was only observed in non-PCOS participants, without any association in PCOS group. Finally, receiver operating characteristic (ROC) curve analysis demonstrated that serum SHBG levels on hCG-day could be used as a predictor of ovarian response in the non-PCOS group (P=0.0015), but not in the PCOS group (P=0.5142).
Limitations, reasons for caution: This study did not investigate the relationship between serum SHBG level and pregnancy outcomes due to the frozen embryo transfer protocol. Secondly, we limited the treatment protocol, age, AMH and other indicators in order to control the interfering factors. Thus, the relationship between SHBG and these factors can't be studied.
Wider implications of the findings: This study provides a means to reassess the clinic value of using serum SHBG levels, especially before COH and on the hCG-day as an ovarian response predictor in non-PCOS patients. Moreover, it also improves our understanding of the intrinsic mechanisms of folliculogenesis and the difference between PCOS and non-PCOS patients.
Trial registration number: N/A

P-559 The circadian clock gene functions as a novel therapeutic target for constant darkness-induced insulin resistance and hyperandrogenism of polycystic ovary syndrome

S. Li[1], J. Zhai[1], W. Chu[1], X. Geng[1], Z.J. Chen[1], Y. Du[1]

[1]Ren Ji Hospital- School of Medicine- Shanghai Jiao Tong University, Center for Reproductive Medicine, Shanghai, China

Study question: What are the mechanisms underlying metabolic and reproductive dysfunction caused by arrhythmic circadian clock and their involvement in polycystic ovary syndrome (PCOS)?
Summary answer: The arrhythmic expressions of circadian clock genes due to constant darkness induced the metabolic and reproductive hallmarks of PCOS in rats.
What is known already: As a complex endocrine condition characterized by oligo/anovulation, high androgen levels and polycystic ovaries, PCOS is closely associated with genetic susceptibility, and environmental risk factor plays an important role in the expression of those genetic traits. Environmental toxins, diet and nutrition, socioeconomic status, and geography are suggested to be potentially involved in the etiology, prevalence, and modulation of PCOS phenotypes. Together environmental factors with genetics contribute to the complicated pathology of PCOS. As a strong environmental risk factor, circadian clock is essentially involved in various diseases. However, the functional role of circadian clock in PCOS remains to be elaborated.
Study design, size, duration: We tried to elaborate the influence of altered circadian clock gene expression caused by constant light or darkness exposure on traits of PCOS in rats and to explore the effects of 5 different rescue treatments. We utilized HepG2 cells and KGN cells to verify the molecular mechanisms. Additionally, human leukocytes and serum of PCOS (n=20) and non-PCOS (n=20) patients were collected to measure expressions of circadian clock genes and other target genes.
Participants/materials, setting, methods: The influence of circadian disruption on hallmarks of PCOS in rats and the effects of rescue treatments were evaluated by estrus cycle detection, ovary morphology, hormone and metabolic factor measurements. And the expressions of circadian clock genes in different rat models and patients were measured with quantitative PCR and western blot. The roles of circadian genes in hyperinsulinemia, hyperandrogenism and apoptosis of ovarian granuloma cells were investigated with small interfering RNA-mediated knockdown and plasmids-mediated overexpression.
Main results and the role of chance: After exposure to constant darkness, decreased brain and muscle ARNT-like protein 1 (BMAL1) promoted insulin resistance *via* glucose transporter 4 (GLUT4), and decreased period (PER) 1 and PER2 promoted androgen excess *via* insulin-like growth factor-binding protein 4 (IGFBP4) and sex hormone binding globulin (SHBG) in the liver. Hyperinsulinemia and hyperandrogenism shared a bidirectional link promoting aberrant expression of circadian genes and inducing apoptosis of ovarian granuloma cells. Notably, the altered expressions of circadian clock genes in darkness-treated rats matched those of PCOS patients. Furthermore, melatonin treatment relieved the hyperinsulinemia and hyperandrogenism of darkness-treated rats *via* BMAL1, PER1, and PER2. Restoring normal light/dark exposure for 2 weeks reversed these conditions *via* BMAL1.
Limitations, reasons for caution: We couldn't compare circadian genes in liver or adipose from women with/without PCOS due to the scarcity of samples. Although the adipose of rats kept in continual darkness exhibited abnormal BMAL1 expression, we did not conduct experiments to account for the unchanged expression in P-AKT pathway under the same conditions.
Wider implications of the findings: Our findings provide a theoretical basis for illustrating the critical function of circadian clock genes, especially *BMAL1*, *PER1*, and *PER2* in PCOS, which might aid the development of feasible preventive and therapeutic strategies for PCOS in women with biorhythm disorder.
Trial registration number: not applicable

P-560 Effect of Ovarian Stimulation on IUI Outcomes - Managing Expectations

B. Wusk[1], F. Roth[1], V. Hungerbuehler[1]

[1]fiore lab, Institute of Reproductive Medicine, St. Gallen, Switzerland

Study question: IUI - natural vs FSH stimulation: how much affects an individual medication the outcome - a single-center, retrospective database analysis over 11 years.
Summary answer: Compared to natural cycles, the application of individual FSH regimen led to a significant increase of 32% of βHCG-positive pregnancies (10.3% vs 13.6%, p≤0.001).
What is known already: A number of contributing factors during ART lead to the concern that children conceived by ART might be exposed to increased health risks as compared to naturally conceived children.
IUI is a simple and non-invasive technique with minimal monitoring and risks. It can be performed without expensive infrastructure with a reasonable success rate within a few cycles in most centers. IUI is undoubtly a more cost effective and patient-friendly strategy than ART (Pennings and Ombelet 2007).
Study design, size, duration: A single-center, retrospective database analysis over 11 years (2008-2019). Exclusion criteria were: HIV, AID, cryopreserved sperm, leucospermia. From a total of n=7164 IUI cycles, 6148 (85.8%) were included in the study . The average IUI attempt per couple was 2.9 (2097 couples with 6148 cycles).
Participants/materials, setting, methods: The study population represented daily clinical IUI practice. Average age of the female population has been:

34.0+4.6 years. Administration or not of FSH has been expressed as yes or no (68% vs 32%). Initial progressive motility (59.8±16), increased after gradient density preparation by 32% (78.4±13).
Main results and the role of chance: From 2008-2019, our overall IUI pregnancy rate (βHCG+) was 12.6% per cycle. For natural cycles, the pregnancy rate per heterologous insemination was 10.3% (n=201/1946), whereas for stimulated IUI cycles the pregnancy rate (573/4202; 13.6%) was significantly increased by 3.3% (p≤0.001).
Limitations, reasons for caution: Since we used center specific sperm preparation procedures (density gradient preparation, constant temperature setting of 34.5°C, HEPES buffer) data comparison with other studies are limited.
Wider implications of the findings: Here, we add substantial information how FSH stimulation can affect the outcome of individual IUI cycles and could assist in couple counselling and planning.
Trial registration number: not applicable

P-561 The mental health of women with polycystic ovary syndrome: a systematic review and meta-analysis

C. Chan[1,2], X. Yin[2], Y. Ji[2], C. Chan, Lai Wan[2]

[1]University of Hong Kong, Centre of Assisted Reproduction and Embryology, Pokfulam, Hong Kong ;
[2]The University of Hong Kong, Department of Social Work and Social Administration, Hong Kong, China

Study question: How is the generalized mental health status of women with PCOS?
Summary answer: The mental health situation of PCOS patients was quite worrisome, which has highlighted the importance of developing psychological health care interventions for them.
What is known already: PCOS has been proposed to be associated with several mental health problems, including somatic symptoms, anxiety, depression, body dissatisfaction, and eating disorders, diminished sexual satisfaction, and lowered health-related quality of life, etc.
Study design, size, duration: A systematic review and meta-analysis of published literature comparing the mental health of women with and without PCOS. Ten English and Chinese databases were searched up to 12/31/2018. 46 studies, including 30,989 participants (9,265 women with PCOS and 25,638 controls), were qualified for review according to the inclusion criteria.
Participants/materials, setting, methods: Random effect models were introduced, and subgroup analysis, sensitivity test, and meta-regression were carried out to determine the source for heterogeneity among studies.
Main results and the role of chance: Twenty-eight studies reported depression symptoms, 22 studies were on anxiety, 16 studies showed QoL status, 12 studies were about sexual dysfunction, five on emotional distress, four on binge eating, and four on somatization. Women with PCOS reported significantly higher depression (SMD=0.64; 95%CI: 0.50-0.78), anxiety (SMD=0.63; 95%CI: 0.50-0.77), lower QoL (SMD=-0.55; 95%CI: -0.71 to -0.39), and not significant sexual dysfunction (SMD=-0.23; 95%CI: -0.51 to 0.04). Studies from different countries, adopting various diagnosis criteria, using diverse instruments, as well as in different years, have reported heterogenetic results. Women with PCOS in China reported a larger effect size of depression and anxiety than patients from other countries.
Limitations, reasons for caution: Although subgroup analysis, meta-regression, and sensitivity tests were carried out to determine the source for high heterogeneity, only 81.63% of the heterogeneity can be explained by this study. There were still unknown sources for heterogeneity among included studies on the depression of PCOS patients.
Wider implications of the findings: PCOS patients suffer from depression, anxiety, and experience a lower QoL, whereas their sexual function is not distinct from that of healthy control. Studies using different instruments, from different countries, and adopting different diagnosis criteria, reported heterogenetic results. PCOS patients in China reported a high level of depression and anxiety.
Trial registration number: N/A

P-562 Inhibin A - a promising predictive parameter of oocyte maturity in ovarian stimulation for IVF/ICSI

B. Lawrenz[1], L. Depret Bixio[2], C. Coughlan[1], C. Andersen[3], B. Kalra[4], G. Savjani[4], L. Melado[1], H. Fatemi[1], A. Kumar[4]

[1]IVIRMA Abu Dhabi, IVF department, Abu Dhabi, United Arab Emirates ;
[2]IVIRMA Abu Dhabi, Statistical department, Abu Dhabi, United Arab Emirates ;
[3]University of Copenhagen, Laboratory of Reproductive Biology- The Juliane Marie Centre for Women- Children and Reproduction-, Copenhagen, Denmark ;
[4]Ansh Labs, Hormonal laboratory, Webster, U.S.A.

Study question: To evaluate the role of Inhibin A as a potential predictive marker of oocyte maturity in ovarian stimulation cycles for IVF / ICSI
Summary answer: On the day of final oocyte maturation, Inhibin A correlates better with the number of retrieved and mature oocytes as compared to E2
What is known already: Monitoring of ovarian stimulation for IVF/ICSI by transvaginal ultrasound (TVUS) and measurement of E2-levels is critical for the planning of final oocyte maturation and oocyte retrieval. E2-levels are supra-physiological due to multifollicular growth and do not facilitate an accurate determination of oocyte maturity. In contrast to E2, Inhibin A levels increase when a minimum follicular size of 12-15mm is attained , the minimal follicular size required to retrieve a mature oocyte . Therefore, the combination of Inhibin A measurement and TVUS may present a more reliable predictive parameter of oocyte maturity as compared to E2 plus TVUS.
Study design, size, duration: Prospective observational study, performed from September 2018 to January 2019, including data of 145 patients recorded at the beginning of ovarian stimulation and 136 patients on the day of final oocyte maturation.
Participants/materials, setting, methods: Patients with primary / secondary infertility and an indication for IVF/ICSI, undergoing ovarian stimulation in a GnRH-antagonist-protocol, were included. On day 2 / 3, AFC was recorded and blood samples were taken.Monitoring of the stimulation was performed according to clinical routine and blood samples were repeated on the day of final oocyte maturation. For measurement of E2, Inhibin A and Inhibin B, samples were thawed and analysed with the same batch of reagents.
Main results and the role of chance: Hormonal results and ovarian stimulation parameters from the IVF-treatments were available from 145 patients at stimulation start (day 2/3) and from 136 patients on the trigger day. 9 patients did not undergo oocyte retrieval. Patient characteristics include (mean ± SD): age 35.4 ± 6.5 years, BMI 28.2 ± 4.8 kg/m², infertility duration 3.9 ± 3.0 years, previous stimulations 3.1 ± 4.2. Correlations were calculated using Pearson´s (ρ) coefficient and probability values (p-value).

On the trigger day, a strong correlation (Pearson´s Coefficient) was found between the total follicle number and Inhibin A / E2 (ρ 0.78 / 0.71) and between the number of follicles ≥ 15 mm and Inhibin A (ρ 0.72). Pearson´s Coefficient showed a strong correlation between Inhibin A and the number of retrieved and mature oocytes (ρ 0.82 / 0.77), whereas E2 had a moderate correlation (ρ 0.69 / 0.69) respectively, for these parameters.
The area under the curve (AUC) for Inhibin A as a predictor for ≥ 10 mature oocytes was ρ 0.91 (CI95% [0.87 ; 0.96]) and ρ 0.84 (CI95% [0.7769 ; 0.9124]) for E2. The optimal predicted threshold level of Inhibin A for ≥ 10 mature oocytes was 668.1 pg/mL (sensitivity=88.0%, specificity=82.0%).
Limitations, reasons for caution: The limitation of the study is that the decision, when to administer medication for final oocyte maturation, was based on E2-level and follicle size, as the Inhibin A results were not available and therefore not used as a decision tool in this setting.
Wider implications of the findings: As Inhibin A on the trigger day correlates better as compared to E2 with the number of follicles ≥ 15 mm and the number of retrieved and mature oocytes, Inhibin A in combination with ultrasound monitoring of follicular development may present a promising tool to facilitate planning of oocyte retrieval.
Trial registration number: clinicaltrials.gov., number NCT03607409

P-563 Intra-Uterine Insemination + Controlled ovarian hyperstimulation versus In Vitro Fertilization in Unexplained Infertility: A systematic review and meta-analysis

A. Nandi[1], R. Gangopadhyay[2], D. White[1], T. El-Toukhy[1]

[1]Guy's and St Thomas' Hospital NHS Trust, Assisted Conception Unit, London, United Kingdom ;
[2]Watford General Hospital- Hertfordshire- UK, Department of Obstetrics and Gynaecology, Wembley, United Kingdom

Study question: What is the relative efficacy of IUI+COH compared with IVF in couples with unexplained infertility?

Summary answer: Results showed a higher success rate following IVF compared to IUI+COH, particularly in older women, but no difference in younger women.

What is known already: Unexplained infertility is a diagnosis of exclusion when all standard fertility investigations, namely tests of ovulation, tubal patency and semen analysis, are normal. The two most applied treatment options are intra-uterine insemination and IVF. However, there is an ongoing debate on the effectiveness of the treatments and the United Kingdom (UK) National Institute for Health and Care Excellence (NICE) fertility guidelines recommend IVF treatment rather than IUI after 2 years of expectant management. We sought to conduct a systematic review of existing evidence to assess the relative efficacy of the two treatments.

Study design, size, duration: We searched Medline, Embase, CIHNL, Pscy Info, and Cochrane Library from 1980 till November 2019. Only RCTs comparing IUI + COH (using either clomiphene citrate or injectable gonadotropins or both) with IVF with female patients aged 18 - 43 years and diagnosed with unexplained infertility were included for the systematic review.

Two authors reviewed citations from primary search independently and any disagreement was resolved by mutual discussion and consultation with a third author.**Participants/materials, setting, methods:** The titles and abstracts were scrutinised to identify relevant articles. The full texts of all potentially relevant articles were retrieved and reviewed to identify articles, which fulfil the study inclusion criteria. Study characteristics including study type and setting, inclusion criteria, recruitment procedure, intervention and outcome data were extracted. All included studies were then assessed for trial quality as per Cochrane guidelines. From each primary study, data were pooled and analysed using Revman software 5.2 versions.

Main results and the role of chance: In total eight RCTs were included. The quality of evidence was moderate to low quality due to inconsistency across the trials and imprecision. The pooled result showed that IVF was associated with a statistically significant higher live birth rate (RR 1.53, 95% CI 1.01 – 2.32, P <0.00001 I^2 = 86%) with no significant difference in multiple pregnancy rate or OHSS rate. Sensitivity analysis based on women's age and a history of previous IUI or IVF treatment showed no significant difference in the live birth rates (RR 1.01, 95% CI 0.88 -1.15, I^2 = 0%, 3 RCTs) between the two treatments in treatment-naïve women younger than 38 years. In women over 38 years, the live birth rates were significantly higher in the IVF group (RR 2.15, 95% CI 1.16 – 4.0, I^2 = 42%, 1 RCT).

Limitations, reasons for caution: The main limitation encountered in this review was the considerable clinical and methodological heterogeneity present among the primary studies, including differences in the definition of unexplained infertility, study design and inclusion criteria as well as treatment protocols used for IUI+COH and IVF.

Wider implications of the findings: Our study included only direct comparisons between IUI+COH and IVF as they are the most commonly applied treatment and showed overall higher success with IVF but no difference in young women <38 years. This will certainly enable clinicians to individualise the care to these couples.

Trial registration number: not applicable

P-564 Agonist vs antagonist administration: is there an age-related difference? A retrospective analysis

F. Roth[1], **B. Wusk**[1], **V. Hungerbuehler**[1]

[1]*FIORE, IVF Lab, St. Gallen, Switzerland*

Study question: Agonist vs antagonist: Is there a best practice/algorithm to suggest for a specific age group of the female idiopathic patient?

Summary answer: In the age group of ≥35 years, a significantly increased pregnancy rate (βHCG+) for the agonist protocol has been observed.

What is known already: Controlled ovarian hyperstimulation (COH) is a fundamental step in ART. GnRH antagonist stimulation protocol has shorter duration of treatment and reduced administration of gonadotropins. GnRH agoinst protocol has shown to be better in folliculogenesis and pregnancy rate, despite its costly and time-consuming.

Study design, size, duration: A single-center, retrospetive database analysis of 1538 antagonist- and 422 agonist protocols over a 10y year period (2009-2019) has been analyzed. Female patients have been divided into four age groups: 26-30 years; 31-35 years; 36-40 years and 41-45 years. Excluded were cases of: disovulation, endometriosis and TESE, MESA or cryopreserved sperm.

Participants/materials, setting, methods: Age-dependant comparison of agonist vs antagonist protocols have revealed a slight to distinct increase of pregnancy rates in favor of agonistic stimulation.

age group	βHCG+ (%) agonist	n	βHCG+ (%) antagonist	n	p value
26-30y	53.2	62	52.7	203	ns
31-35y	53.2	188	49.6	470	ns
36-40y	51.6	153	39.9	592	p=0.009
41-45y	42.1	19	23.4	273	p=0.07

Main results and the role of chance: The agonist protocol yields in higher pregancy rates (βHCG+) in females ≥35 years as compared to the antagonist protocol, whereas in age groups ≤ 34 years no differences were observed.

Limitations, reasons for caution: The retrospective character of the analysis limits the statement of a general recommendation.

Wider implications of the findings: Age-related administration of GnRH should be confirmed by prospective, randomized, clinical studies.

Trial registration number: not applicable

P-565 Randomised control trial comparing the effects of myoinositol to metformin on ART outcome in women with PCOS undergoing In-vitro fertilisation (IVF) cycle

K. Rajasekaran[1], **N. Malhotra**[1]

[1]*All India Institute of medical sciences AIIMS, Department of obstetrics and gynaecology, new delhi, India*

Study question: Does Myoinositol in comparison to metformin in infertile PCOS women, improves the ART outcomes, oocyte quality, pregnancy rates and decreases the incidence of OHSS

Summary answer: Myoinositol reduces unsuitable oocytes, improves the menstrual pattern, insulin resistance, hormone profile, quality of embryos, IVF outcomes and clinical pregnancy without significant change in OHSS.

What is known already: Myoinositol and metformin are insulin sensitisers. Metformin reduces testosterone concentrations, optimises IVF outcome, reduces risk of OHSS in PCOS but is associated with gastrointestinal side effects and lactic acidosis resulting in reduced patient's compliance. Myoinositol improves ovarian function, oocyte quality, LH/FSH ratio, reduces serum androgens, improves ovulation and fertility outcome without the side effects of metformin. Myoinositol acts at the level of insulin receptors and improves hyperinsulinemia, positively correlate with quality and maturity of oocytes reducing mean number of immature and degenerated oocytes, quantity of gonadotropins or units of FSH necessary for ovarian stimulation in IVF protocols.

Study design, size, duration: The present study is a randomised controlled trial conducted after the ethical committee approval, in the ART Clinic, department of Obstetrics and Gynaecology, All India Institute of Medical sciences, New Delhi from March 2017 to March 2019. A total of 231 patients infertile PCOS women undergoing IVF cycles were enrolled in the study and asses for eligibility and 102 were randomised and allotted as 50 and 52 in group 1(Myoinositol) and group 2(Metformin) respectively.

Participants/materials, setting, methods: Recruited patients received myoinositol 2gm twice daily(group1) and metformin 850mg twice daily(group 2). Pre and post treatment clinical(menstrual pattern,BMI), hormonal profile(LH,FSH,Testosterone, prolactin, AMH), biochemical parameters(HOMA IR, fasting glucose, insulin) and side effect profile assessed. After 3 months of therapy, patients were recruited for IVF cycles involving controlled ovarian stimulation, cycle monitoring, oocyte recovery, insemination of oocytes and follow up with fertilisation, cleavage, transfer of good grade cleavage embryos or blastocysts pregnancy outcomes and OHSS incidence

Main results and the role of chance: Myoinositol had significant fertilisation rate(p<0.001), embryos retrieved(p<0.001), cleavage rate(p 0.008), grade 1 embryos(p 0.042) with no significant difference in implantation

rate(p 0.537),number of oocytes retrieved(p0.097),metaphase II oocytes(p 0.176) and Grade1 oocyte(p 0.204). Embryo transfer cancelled was significantly high in metformin group(p 0.035). Starting dose of FSH, total dose of FSH and duration of gonadotropin stimulation were comparable.

Clinical pregnancy rate was significantly high in Myoinositol group 36.0% (18/50); 95% CI 18.0(0.9,35.0) p value 0.043) compared to metformin(18% (9/50)). The spontaneous pregnancy rate were comparable, but Group 1 had higher number of patients conceived spontaneously(13,26%) compared to Group 2(6,12%) (p value 0.074).The total number of IVF pregnancy rates (p value 0.715), total number of IVF pregnancy rate per number of IVF cycles (p value 0.314) and the total number of IVF pregnancy rate per embryo transfer (p value 0.368) were also comparable between the Groups. Incidence of OHSS was 10% (5/50) and 20% (10/50) in Group 1 and Group 2 respectively, comparable between both Myoinositol and Metformin Group (p value 0.001).
Myoinositol group had increased regularity in menstrual pattern(0.001) and improvement in fasting insulin (p 0.001),HOMA IR (p 0.001),Serum AMH(p 0.001) and Serum SHBG(p 0.032) thereby suggesting decreased insulin resistance.

Limitations, reasons for caution: Most patients with PCOS recruited in our study were brittle PCOS, with high LH, AMH and hyperandrogenemia with sub-optimal response during ovulation induction and IUI. In IVF cycles only fresh embryo transfer and not FET cycles were analysed. These were attributed to be the reason for lower Pregnancy outcomes.

Wider implications of the findings: Myoinositol in comparison to metformin in infertile PCOS women has better IVF outcome and oocyte quality, however to make these results more robust, a large multi-centric trial with larger sample size and longer duration is necessary.

Trial registration number: CTRI/2018/05/014196

P-566 A randomised, assessor-blind, controlled phase 3 non-inferiority trial assessing the efficacy and safety of individualised follitropin delta dosing regimen in Japanese IVF/ICSI patients

O. Ishihara[1], M. Kitamura[2], J.C. Arce[3], X. Japanese Follitropin Delta Phase 3 Study Group[4]

[1]*Saitama Medical University, Department of Obstetrics & Gynaecology, Iruma-gun- Saitama, Japan ;*
[2]*Ferring Pharmaceuticals, Development, Tokyo, Japan ;*
[3]*Ferring Pharmaceuticals, Reproductive Medicine & Maternal Health, Copenhagen, Denmark ;*
[4]*On behalf of the participating sites, Fertility clinics, Japan, Japan*

Study question: To establish the efficacy and safety of controlled ovarian stimulation with follitropin delta individualised fixed-dose regimen versus follitropin beta conventional dosing in Japanese women.

Summary answer: Individualised treatment with follitropin delta versus follitropin beta resulted in non-inferiority in number of oocytes retrieved, comparable clinical pregnancy rates and significant reduction of OHSS.

What is known already: The follitropin delta dosing regimen stratifies patients according to response potential and applies an individualised dose based on an ovarian biomarker (AMH) and a patient characteristic (body weight). This approach has in previous clinical trials conducted outside of Japan been demonstrated to optimise ovarian response and reduce OHSS risk without compromising success rates.

Study design, size, duration: Randomised, assessor-blind, controlled trial conducted in 347 Japanese women, 20-40 years (mean 34.1), undergoing their first IVF/ICSI cycle. Randomisation was stratified by AMH at screening (<15 pmol/L, ≥15 pmol/L). The primary endpoint was number of oocytes retrieved with a prespecified non-inferiority margin (lower limit of 95% confidence interval (LL 95% CI) >-3.0 oocytes). A GnRH antagonist protocol was applied. Clinical pregnancy was assessed 5-6 weeks after single blastocyst transfer. OHSS was classified using Golan's system.

Participants/materials, setting, methods: The follitropin delta (REKOVELLE, Ferring Pharmaceuticals) dose was individualised based on serum AMH and body weight, and was fixed throughout stimulation – AMH <15 pmol/L: daily dose of 12 µg, AMH ≥15 pmol/L: daily dose decreasing from 0.19 to 0.10 µg/kg body weight by increasing AMH (min-max 6-12 µg). Elecsys® AMH, Roche Diagnostics was used. The follitropin beta (FOLLISTIM/PUREGON, MSD) dose was 150 IU/day for the first five days and could thereafter be adjusted.

Main results and the role of chance: The primary objective of the trial was met, as non-inferiority was established for the number of oocytes retrieved for follitropin delta compared to follitropin beta (9.3 vs 10.5; LL 95% CI 2.3). Among patients with AMH <15 pmol/L (40.9%), the proportion of women with <4 oocytes was similar in the two treatment groups (11.6% vs 12.3%). Among patients with AMH ≥15 pmol/L (59.1%), treatment with follitropin delta resulted in significantly ($p<0.05$) fewer patients with ≥15 oocytes (22.0% vs 42.0%) or ≥20 oocytes (8.0% vs 19.0%). Blastocyst transfer was performed for 79.4% and 79.7% in the follitropin delta and follitropin beta groups, respectively, and the clinical pregnancy rate for these women was 31.9% and 29.8%, respectively. The clinical pregnancy rate per started cycle was 25.3% for follitropin delta and 23.7% for follitropin beta. The occurrence of OHSS was reduced to approximately half with follitropin delta compared to follitropin beta, with an incidence of 11.2% vs 19.8% ($p<0.05$) for OHSS of any grade, and 7.1% vs 14.1% ($p<0.05$) for moderate/severe OHSS. Two patients in the follitropin beta group were hospitalised due to OHSS for a duration of 16 and 33 days, respectively, while there were no hospitalisations in the follitropin delta group.

Limitations, reasons for caution: The individualised follitropin delta dosing regimen based on the patient's serum AMH and body weight was developed after completion of a phase 2 trial in non-Japanese women. Based on a subsequent phase 2 trial conducted in Japan, the individualised dosing regimen was evaluated to also be applicable to Japanese women.

Wider implications of the findings: This trial confirms that the ovarian response associated with the individualised dosing of follitropin delta preserves pregnancy rates and results in an improved OHSS risk profile for Japanese IVF/ICSI patients as already observed in non-Japanese women.

Trial registration number: NCT03228680

P-567 Midfollicular gonadotropin dose increases do not appear to change the ovarian response determined by the dose selected at the start of the stimulation

J.C. Arce[1], B. Klein[2]

[1]*Ferring Pharmaceuticals, Reproductive Medicine & Maternal Health, Copenhagen, Denmark ;*
[2]*Ferring Pharmaceuticals, Global Biometrics, Copenhagen, Denmark*

Study question: Can gonadotropin dose increases during stimulation lead to more oocytes or blastocysts compared to maintaining a fixed dose selected based on biomarkers and patient characteristics?

Summary answer: Gonadotropin dose increases in the midfollicular phase do not appear to alter the ovarian response or blastocyst availability defined by the starting dose.

What is known already: Studies have indicated that increasing the gonadotropin dose after the first 5 days (or later) in anticipation of low ovarian response does not rectify the response (van Hooff et al, 1993; Khalef et al, 2002). As follicular recruitment occurs in the late luteal and early follicular phase, such dose increases during the stimulation cycle does not lead to increasing the number of oocytes retrieved.

Study design, size, duration: Post-hoc analysis of a randomised, assessor-blind, controlled trial in 1,326 women, 18-40 years, undergoing their first IVF/ICSI cycle. Patients randomised to follitropin alfa (GONAL-F, Merck) (N=661) received a daily starting dose of 150 IU for the first five days which thereafter could be adjusted by 75 IU. Patients randomised to follitropin delta (REKOVELLE, Ferring Pharmaceuticals) (N=665) received an individualised dose based on AMH and body weight, and the dose was fixed throughout stimulation.

Participants/materials, setting, methods: Investigators were blinded to treatment allocation. The frequency of investigator-requested dose increases on stimulation day 6 was similar in the two treatment groups, with 24.5% (n=162) in the follitropin alfa group and 22.6% (n=150) in the follitropin delta group. The investigator's requests for gonadotropin dose adjustments were only implemented in the follitropin alfa group, while patients in the follitropin delta group remained on the assigned daily dose.

Main results and the role of chance: The follicular development and serum endocrine profile on stimulation day 6 were comparable between the women in the follitropin alfa and follitropin delta groups for whom the investigator recommended to increase the dose. These women had 12.3±5.8 and 12.8±6.7 follicles in the follitropin alfa and follitropin delta groups, respectively, of which there were 4.8±3.6 and 4.2±3.4 follicles ≥10mm. The median [interquartile

range] estradiol was 1509 [898;2264] pmol/L for follitropin alfa and 1228 [762;2076] pmol/L for follitropin delta, while inhibin B was 434 [263;706] pg/mL and 430 [282;637] pg/mL, respectively. Among the women with investigator-requested dose increases, the ovarian response and blastocyst development were comparable between the follitropin alfa patients with dose increases and the follitropin delta patients on a fixed dose. For follitropin alfa and follitropin delta, respectively, these women had 7.4±5.0 versus 7.4±5.1 oocytes retrieved, 2.5±2.4 versus 2.3±2.4 blastocysts, and 1.3±1.7 versus 1.3±1.9 good-quality blastocysts. The dose increases in the follitropin alfa group logically influenced the gonadotropin consumption. The mean daily gonadotropin dose in this sub-population was 196±26 IU (14.4±1.9 µg) follitropin alfa versus 10.2±2.4 µg follitropin delta, with a mean total gonadotropin dose of 1865±607 IU (137±45 µg) follitropin alfa versus 99±26 µg follitropin delta.

Limitations, reasons for caution: Although the population with investigator-requested dose increases was similar in the follitropin alfa and follitropin delta groups, it should be noted that the decision to request an increase of the starting gonadotropin dose was per the investigator's judgement and not based on protocol-specific criteria.

Wider implications of the findings: These findings stress the importance of appropriate dose selection before starting stimulation. The data indicate that increasing the dose during stimulation does not modify the ovarian response that can be obtained with a fixed-dose regimen. Maintaining a fixed-dose regimen throughout treatment is convenient for both the patient and clinic staff.

Trial registration number: NCT01956110

P-568 differences between steroids gene expression in subcutaneous fat of PCOS and non-PCOS pregnant women

N. Emami[1], A. Alizadeh[1], A. Moini[2], M. Shahhosseini[3]

[1]Royan Institute for Reproductive Biomedicine- ACECR- Tehran- Iran, Department of Embryology- Reproductive Biomedicine Research Center, tehran, Iran ;
[2]Royan Institute for Reproductive Biomedicine- ACECR- Tehran- Iran, Department of Endocrinology and Female Infertility- Reproductive Biomedicine Research Center-Breast Disease Research Center BDRC- Tehran University of Medical Sciences- Tehran- Iran- De, ;
[3]Royan Institute for Reproductive Biomedicine- ACECR- Tehran- Iran-, Department of Genetics- Reproductive Biomedicine Research Center- Royan Institute for Reproductive Biomedicine- ACECR- Tehran- Iran- Department of Cell and Molecular Biology- School o,

Study question: Are there differences between gene expression related to steroid metabolism in abdominal subcutaneous AT in PCOS and non- PCOS pregnant women?

Summary answer: PCOS SAT(subcutaneous adipose) showed significantly higher level of those gene incorporated in glococorticoids and mineralocorticoids but not sex steroids genes

What is known already: A series of steroidogenic and steroid-inactivating enzymes have been detected in human adipose tissue. The abnormal conversion of androgen precursors to both active and inactive forms at the receptor level is important for the pathogenic of metabolic diseases found in PCOS..Most recently, it has been suggested that adipose tissue may contain the steroidogenic machinery necessary for the initiation of steroid biosynthesis *de novo* from cholesterol.

Study design, size, duration: . Samples and demographic data were collected from three hospitals in Tehran, Iran. The Subjects were 45 Iranian pregnant women underwent cesarean section; 13 PCOS (poly cystic ovarian syndrome) and 32 were non–PCOS, whose samples from subcutaneous fat of abdomen were taken.Diagnosis of these PCOS subjects is responsibility of the medical practitioner related to Royan Institute.At the time of caesarean section, the surgeon took 3-4 g subcutaneous fat upon entering the abdomen were collected .

Participants/materials, setting, methods: Total RNA extraction and removal of genomic DNA from the adipose tissue were performed using the RNeasy lipid Mini Kit (Qiagen, cat. no: 74004).Twenty nanograms of total RNA was used for cDNA synthesis according to manufacturer's instruction. Messenger RNA quantification was performed by qRT-PCR on the Step-One RT-PCR system (Applied Biosystems, USA).Gene expression data were analyzed using $2^{-\Delta\Delta Ct}$ algorithm to calculate the 15 steroids gene mRNA level relative to the level of GAPDH mRNA level.

Main results and the role of chance: No significant differences were found with respect to age and body mass index (BMI) among non-PCOS and PCOS pregnant women. We detected expression of *17BHSD1*,3,5.7.12., *star*, *CYP11A1*, *CYP17A1*, *CYP21A1*, *CYP19A1*,*3BHSD1-2.11BHSD1-2* in the subcutaneous adipose tissue of pregnant women but 17BHSD2 was undetectable in both groups. PCOS subcutaneous AT showed significantly higher level of *CYP11A1*, *CYP17A1*, *CYP21A1*, *STAR* and *11BHSD1-2* mRNA when compared with the non-PCOS women ($P < 0.05$). There was no significant difference between *CYP19A1*,*3BHSD1-2. 17BHSD*1,3, 5.7.12. mRNA abundance between two group.

Limitations, reasons for caution: Although we collected 48 samples during one year, the small sample size is a limitation. Nevertheless, the strength of the observed differences in aged-matched and BMI-matched subjects suggests that power was not an important issue and that a larger study would be able to detect same findings.

Wider implications of the findings: This is the first report for gene expression profiles in AT of PCOS mothers which opens the road for exploration regarding gene expression related glucocorticoids and mineralocorticoids metabolism in AT for pregnant women suffering from PCOS.

Trial registration number: enter 'not applicable' for non-clinical trials

P-569 A multicenter and multinational non-interventional study exploring the POSEIDON criteria – An analysis of 11042 cycles using real-world data from Brazil, Turkey, and Vietnam.

S. Esteves[1],[2], H. Yarali[3], L. Vuong[4], F. Bento[1], I. Özbek[3], P. Mehtap[3], A.H. Le[5], T. Pham[5], T. Ho[5], P. Humaidan[2,6], C. Alviggi[7]

[1]ANDROFERT, Andrology & Human Reproduction Clinic, Campinas, Brazil ;
[2]Faculty of Health- Aarhus University, Department of Clinical Sciences, Aarhus, Denmark ;
[3]Anatolia IVF, Endocrinology, Ankara, Turkey ;
[4]University of Medicine and Pharmacy at Ho Chi Minh City, Department of Obstetrics and Gynecology, Ho Chi Minh, Vietnam ;
[5]My Duc Hospital, IVF Unit, Ho Chi Minh, Vietnam ;
[6]Skive Regional Hospital, Fertility Clinic, Skive, Denmark ;
[7]University of Naples Federico II, Department of Neuroscience- Reproductive Science and Odontostomatology, Naples, Italy

Study question: What is the cumulative live birth rate per started cycle (CLBR) in low prognosis women stratified according to the POSEIDON criteria?

Summary answer: CLBR varies in POSEIDON groups (48% in G1, 24% in G2, 29% in G3, 10% in G4), impacted by female age and number of oocytes.

What is known already: The POSEIDON criteria have been introduced as a new classification for low-prognosis women undergoing assisted reproductive technology (ART). The low prognosis patient is classified into four groups according to the results of ovarian reserve markers, female age, and the number of oocytes retrieved in previous IVF/ICSI cycles of conventional ovarian stimulation (OS). The low prognosis is supposedly caused by the impact of a decreased number of oocytes, which limits the number of embryos produced. This condition might be aggravated by advanced female age, thus negatively impacting the CLBR per started cycle.

Study design, size, duration: This study analyzed the data of 11,042 consecutive infertile couples who initiated IVF/ICSI treatment in three large Fertility Centers in Brazil, Turkey, and Vietnam between 2015 and 2017. Ovarian reserve was assessed before the cycle by either anti-Mullerian hormone (AMH) or antral follicle count (AFC), or both. The included patients were treated using conventional OS protocols.

Participants/materials, setting, methods: Patients were stratified into five groups based on female age, ovarian markers, and number of oocytes retrieved, including the previously described low prognosis POSEIDON groups (Groups 1-4), and a group of patients not fulfilling the criteria (Non-POSEIDON; NP). The primary outcome was the CLBR per started cycle, including all fresh and frozen-thawed embryo transfers. The secondary outcomes were the number of embryos per group and the prevalence of low prognosis patients in dataset.

Main results and the role of chance: The CLBR of patients fulfilling the POSEIDON criteria was, on average, ~30% lower than NP patients. Younger unexpected poor/suboptimal responders (G1; n=1,480) and expected poor responders (G3; n=852) had a CLBR of ~48% and ~29%, respectively ($p<0.0001$). The CLBRs of older unexpected poor/suboptimal responders (G2; n=1,726) and expected poor responders (G4; n=1,711) were ~24% and ~10%, respectively ($p=0.001$). Overall, the CLBR of NP patients (59.2%; n=5,273) was higher than POSEIDON patients ($p<0.0001$). Younger (G1b; n=1,214) and older

(G2b; n=1,301) unexpected suboptimal responder (4-9 oocytes) patients had a CLBR of ~54% and 28%, respectively, which were higher (p=0.0001) than that of unexpected poor responder (1-4 oocytes) patients (G1a: 23%, n=266; G2a: 12%, n=425). The number of embryos varied among the POSEIDON groups, impacted by the number of retrieved oocytes, and were overall lower than that of NP patients (p<0.0001). POSEIDON patients represented ~50% of all treated patients (Brazil: 64%; Turkey: 55%; Vietnam: 40%), with regional differences primarily owing to the average age of treated women.

Limitations, reasons for caution: Retrospective study design, with different practices in participating centers, might influence cycle cancellation, oocyte yield, number of embryos, and therefore, CLBR. However, our data provide the first multicenter real-world evidence to validate the concept of low prognosis –in terms of CLBR per started cycle– in POSEIDON patients.

Wider implications of the findings: The CLBR per cycle in POSEIDON patients is affected by both female age and number of oocytes retrieved. Thus, in addition to aid in patient counseling, the POSEIDON criteria may be used to guide clinical management with a specific focus on therapeutic strategies focused on optimizing the oocyte yield.

Trial registration number: NA

P-570 Levothyroxine and subclinical hypothyroidism in patients with recurrent pregnancy loss

H. Yoshihara[1], M. Sugiura-Ogasawara[1], S. Goto[1], K. Tamao[1]

[1]Nagoya City University Graduate School of Medical Sciences, Obstetrics and Gynecology, Nagoya, Japan

Study question: It was unclear whether subclinical hypothyroidism (SCH) affects subsequent live births and whether levothyroxine is effective in improving the live birth rate in patients with recurrent pregnancy loss (RPL).

Summary answer: SCH did not affect live births and levothyroxine did not improve the live birth rate.

What is known already: It has been reported that the frequency of miscarriage was significantly higher in women with SCH as compared with women on thyroid-stimulating hormone (TSH) ≦ 2.5 mIU/L and that intervention reduces the adverse pregnancy outcome in antithyroid peroxidase antibody (TPO)-positive women with SCH.

One study showed that out of 286 patients with two or more early losses, 19% had SCH, however, that there was no difference in the subsequent live birth rate between patients with SCH and euthyroidism (69% and 74%), either with or without treatment for SCH (71% and 67%).

Study design, size, duration: An observational cohort study was conducted at Nagoya City University Hospital between 2010 and 2019. The study included 1566 pregnancies of 1120 patients with a history of 2 or more pregnancy losses.

Participants/materials, setting, methods: 4D-Ultrasound, hysterosalpingography, chromosome analysis for both partners, antiphospholipid antibodies and blood tests for TSH, free thyroxine (FT4) and diabetes mellitus were performed before a subsequent pregnancy. SCH was defined as having a serum TSH > 2.5 mIU/L with a normal level of FT4. Live birth rates were compared between SCH and euthyroid patients treated with and without levothyroxine after excluding patients with an abnormal chromosome in either partner and those with a uterine anomaly.

Main results and the role of chance: The prevalence of SCH was 15.2 % (170/1120). Subsequent live birth rates were 79.2 % (42/53) for the levothyroxine group, 67.6 % (73/108) for the untreated SCH group and 69.6 % (606/871) for the euthyroid group. After excluding miscarriages with embryonic aneuploidy, chemical pregnancies and ectopic pregnancies, live birth rates were 91.3 % (42/46) for the levothyroxine group, 90.1 % (73/81) for the untreated SCH group and 90.9 % (606/667) for the euthyroid group. The live birth rates per pregnancy were 93.5% (58/62), 88.9% (96/108) and 90.3% (833/922), respectively. There was no significant difference in the live birth rate among the three groups.

Limitations, reasons for caution: The sample size of SCH patients was relatively small. Further RCT is necessary to confirm these results.

Wider implications of the findings: The measurement of TSH and FT4 might not be necessary for the screening of patients with RPL who have no features of hypothyroidism.

Trial registration number: not applicable

P-571 Is ovarian response related to adverse perinatal outcomes after fresh embryo transfer in GnRH antagonist downregulated IVF/ICSI treatment cycles?

E. Bardhi[1], C. Blockeel[1], W. Cools[2], S. Santos-Ribeiro[3], A. Racca[1], S. Mackens[1], M. De Vos[1], N. Polyzos[4], B. Popovic-Todorovic[1], M. De Brucker[1], H. Tournaye[1], P. Drakopoulos[1]

[1]Uz Brussel, Centrum voor Reproductieve Geneeskunde CRG, Avenue du Laerbeek 101- 1090 Jette, Belgium ;
[2]Uz Brussel, Interfaculty Center Data processing & Statistics ICDS, Avenue du Laerbeek 101- 1090 Jette, Belgium ;
[3]IVI-RMA Lisboa-, Instituto Valenciano de Infertilidade, Lisboa, Portugal ;
[4]Dexeus University Hospital- Salud de la Mujer Dexeus, Department of Reproductive Medicine, Barcelona-, Spain

Study question: Is there an association between ovarian response, as expressed by the number of oocytes retrieved, and adverse perinatal outcomes?

Summary answer: Ovarian response is not related to adverse perinatal outcomes.

What is known already: Several studies have found that singleton IVF pregnancies are at higher risk for adverse perinatal outcomes compared to naturally conceived ones. Possible explanations for this include infertility itself, embryo specific epigenetic modifications due to embryo culture and vascular endothelial dysfunction associated with ovarian ageing. However, one of the most accredited hypothesis for these findings, blames the altered endometrium function resulting from the non-physiological endocrine milieu that follows ovarian stimulation, which in turn can be related to ovarian response. Up to date, the safety of ART in terms of obstetrical and neonatal outcomes still remains under investigation.

Study design, size, duration: This was a retrospective, single-center cohort study including infertile women attending a tertiary university center from January 2009 to December 2015.

Participants/materials, setting, methods: All women who underwent their first ovarian stimulation cycle in a GnRH antagonist protocol, with a fresh embryo transfer that resulted in a singleton live birth, were eligible for this study. Patients were categorized into four ovarian response groups according to the number of retrieved oocytes: 1–3 (category I), 4–9 (category II), 10–15 (category III), or > 15 oocytes (category IV).

Main results and the role of chance: The overall number of patients analyzed was 964: 67 in category I, 450 in category II, 269 in category III and 178 in category IV.

Neonatal weight (in grams) was comparable between all groups (3222 ± 607g vs. 3254 ± 537g vs. 3235 ± 575g vs. 3200 ± 622g, *P*=0.85) as well as the term of delivery (38.4 ± 2.4 vs 39 ± 1.7 vs 39 ± 2.0 vs 38.6 ± 2.4 weeks, *P*=0.5). Birth weight z-scores and neonatal gender did not differ among the four oocyte categories (*P*=0.9 and *P*=0.17, respectively). The incidence of preterm birth and low birth weight was also comparable across the different oocyte categories (*P*=0.127 and *P*=0.19, respectively). Finally, gestational diabetes, hypertension disorders of pregnancy, placenta previa, intra-uterine growth restriction, premature rupture of membranes, hospitalization for threatened preterm labor, delivery mode, APGAR scores at 1, 5 and 10 minutes and major fetal malformations did not differ significantly among ovarian response categories either. Multivariate regression analysis (adjusting for female age, body mass index (BMI), initial stimulation dose, type of insemination procedure), number of embryos transferred and embryo stage at transfer, revealed that the number of oocytes was not associated with neonatal birth weight.

Limitations, reasons for caution: This is a large observational study based on retrospective data collection. Despite our robust methodological approach, the presence of biases related to retrospective design cannot be excluded.

Wider implications of the findings: The data of our study are reassuring and refute potential fears of a negative impact from enhanced ovarian response on neonatal outcomes. Future, larger investigations are needed to validate these results.

Trial registration number: not applicable

P-572 17β-oestradiol/nomegestrol pre-treatment for ovarian stimulation GnRH antagonist protocols do not affect clinical pregnancy rates.

M. Samama[1,2,3], R.C.P. Piscopo[4], F. Ikeda[2,3], V.H. Kussumoto[2], A. Sartor[3,5], L. Semião-Francisco[3], J. Ueno[2,3], Z. Jarmy_Di Bella[6]

[1]Escola Paulista de Medicina UNIFESP-EPM- Department of Gynecology- Sao Paulo- Brazil., post-doctoral research trainee Department of Gynecology, Sao Paulo / SP, Brazil ;
[2]Gera Institute, Post-graduation, São Paulo, Brazil ;
[3]Gera Institute, Clinical, São Paulo, Brazil ;
[4]Gera Institute, Post Graduation, São Paulo, Brazil ;
[5]Gera Institute, Post-graduation, São Paulo, British Virgin Islands ;
[6]Escola Paulista de Medicina UNIFESP-EPM- Department of Gynecology- Sao Paulo- Brazil., Professor Head of the Division of Department of General Gynecology, São Paulo, Brazil

Study question: Whether pre-treatment with an oral contraceptive pill with natural oestrogen, 17β-oestradiol/nomegestrol, has effects on hormonal, embryological and clinical outcomes for women undergoing assisted reproductive techniques.
Summary answer: 17β-oestradiol/nomegestrol favor top quality embryo formation and was not associated with deleterious effects on biochemical and clinical pregnancy.
What is known already: Pre-treatments with combined oral contraceptive pill (COCP) suppress the woman's own hormones production and could improve ovarian response to the hormone therapy in *in vitro* fertilization cycles. In the GnRH antagonist protocols with synthetic oestrogen combined oral contraceptive pill pre-treatment, the rate of live birth/ongoing pregnancy was lower than with no pre-treatment.
Study design, size, duration: Cohort study in an assisted reproduction techniques (ART) post-graduation program setting and no pre-treatment patients set in an assisted reproductive center in Sao Paulo, Brazil, which ran from 2017 to 2019, with a total number of patients of 130.
Participants/materials, setting, methods: Three groups were analyzed: no pre-treatment patients were recruited from the ART center, patients from the post-graduation program received natural oestrogen combined oral contraceptive pill and were compared to a synthetic COCP group, whose data was analyzed retrospectively as a reference population. Patients underwent pre-treatment with COCP before GnRH antagonist ovarian stimulation protocol for *in vitro* fertilization (IVF). Statistical analysis was conducted using generalized linear models or binomial regression. Significance was defined if $p<0.05$.
Main results and the role of chance: Significant effects of treatment group were present in the number of embryos (no pre-treatment mean=2.3; 17β-oestradiol/nomegestrol group mean=3.41; p=0.006) and number of top-quality embryos (day 3) (no pre-treatment group mean=1.3; 17β-oestradiol/nomegestrol mean=2.64; p=0.031). The mean number of mature oocytes was significantly higher in natural oestrogen COCP compared to synthetic COCP, when the analysis was controlled for the duration of COCP treatment (17β-oestradiol/nomegestrol group mean=6.28 and ethinylestradiol/gestodene group mean=4.34; p=0.014). However, there were no significant differences in the mean number of mature oocytes between 17β-oestradiol/nomegestrol group and no pre-treatment group (means=6.284 and 5.56, respectively; p=1). 17β-oestradiol/nomegestrol was not statistically associated with deleterious effects on biochemical pregnancy (odds ratio=0.32, CI 95%=0.074–1.392; p=0.129). Ethinylestradiol/gestodene lowered chances of having positive biochemical pregnancy when compared to the no pre-treatment group (odds ratio=0.138, CI 95%=0.028–0.694; p=0.016). 17β-oestradiol/nomegestrol was not associated with negative clinical pregnancy outcome (odds ratio=1.714, CI 95%=0.339–8.676; p=0.515). Mean total dose of follicle stimulating hormone administered for each treatment group was 2349.02 in 17β-oestradiol/nomegestrol group, 2259.24 in no pre-tretment group and 2859.21 for ethinylestradiol/gestodene.
Limitations, reasons for caution: There might be a possible bias of unaccounted variables, such as the number of blastocysts transferred, considering that in the clinical setting in which the study took place, patients are preferentially programmed to transfer at day 3, and thus possibly altering the ratio of total blastocyts to embryos transferred.
Wider implications of the findings: Natural oestrogen COCP was not associated with deleterious embryological or clinical outcomes, and significantly improved the number of embryos and top-quality embryos. Therefore, 17β-oestradiol/nomegestrol prescription might benefit IVF cycle scheduling, and possibly better synchronized follicular growth and future positive clinical pregnancy with cryopreserved embryos obtained from 17β-oestradiol/nomegestrol patients.
Trial registration number: Not Applicable

P-573 Anti-Müllerian hormone (AMH) lower reference values observed in a population of Indian women compared to French women, using an automated AMH assay

B. Mougin[1], J. Kaur[2], P. Bourron[1], S. Bellvert[3], M. Loubet[3], N. Mahajan[2]

[1]Biomérieux SA, Medical Affairs, Marcy l'Etoile, France ;
[2]Mother & Child Hospital, Fertility Clinic, New Delhi, India ;
[3]Biomérieux SA, Clinical Affairs, Marcy l'Etoile, France

Study question: How much do reference VIDAS® AMH values differ for women between India and France?
Summary answer: Significantly lower serum AMH levels were observed for women aged 20 to 44 living in India compared to women living in France.
What is known already: Serum AMH testing is routinely performed in female patients for the assessment of the ovarian reserve. Several published studies have reported differences in age-specific AMH reference values in various countries. Reference intervals have been previously defined for VIDAS® AMH, in populations living in France and in India.
Study design, size, duration: This study compared the age-specific reference VIDAS® AMH values defined during dedicated studies performed in France and in India, for the 20-44 year age range.
Participants/materials, setting, methods: Healthy fertile women with no endocrine or gynaecological disorders were enrolled in the studies conducted in India and France. Serum AMH concentrations were measured using the bioMérieux VIDAS® AMH assay, and comparative analyses were done for the 20-44 year age range, for 748 Indian and 356 French women. Descriptive statistics and linear regression modelling after log transformation were used to estimate the differences.
Main results and the role of chance: Overall lower median AMH values were observed for India with calculated differences of 1.52, 0.50, 1.59, 0.54 and 0.19 ng/mL for the five age classes covering the 20-44 year age range, respectively. The equations for the linear regression models were the following: log(AMH)=-0.0358*(age)+1.4916 for France and log(AMH)=-0.0396*(age)+1.4006 for India. These results confirm previous data reporting lower serum AMH values in women living in India compared to women living in France.
Limitations, reasons for caution: This study focused on women within the 20-44 year age range. Despite selected inclusion criteria and quite large size of the two study populations, more studies are necessary to further characterize the lower serum AMH levels observed in Indian women, in populations representing the regional and ethnical diversity for India.
Wider implications of the findings: These results are in line with CLSI guideline recommendations to check local reference intervals, that particularly makes sense for AMH. They should also stimulate more studies to cover the population diversity worldwide, and to contribute to the adoption of the appropriate usage of this biomarker for management of infertile women.
Trial registration number: Not applicable

P-574 Influence of cross-sex hormone therapy in trans*persons on laboratory profile and BMI

K. Feil[1], A.L. Zippl[1], E. Bürstmayr[1], B. Böttcher[1], K. Winkler-Crepaz[2], B. Toth[1]

[1]Medical University of Innsbruck, Gynecological Endocrinology and Reproductive Medicine, Innsbruck, Austria ;
[2]Paracelsus Medical University Salzburg, Gynecology and Obstetrics, Salzburg, Austria

Study question: What influence has cross-sex hormone therapy (CHT) on concentration of gonadotropins, sex steroids, liver enzymes, triglyceride, hemoglobin and body mass index (BMI)?
Summary answer: Cross-sex hormone therapy seemed to be safe within the study population. Significant changes were present in trans*men for SHBG and hemoglobin.
What is known already: For many trans*persons CHT is the milestone of a life as a member of the desired sex. For this purpose, male and female sex steroids are applied, which can also lead to major side effects.
Study design, size, duration: This retrospective study was performed at our Department between 2005 and 2017. The concentration of gonadotropins

and sex steroids as well as liver enzymes, triglycerides, hemoglobin and body mass index were analysed before and three, six, twelve and twenty-four months after start of CHT. Demographic data were also evaluated.
Participants/materials, setting, methods: In total 84 patients (33 trans*-women and 51 trans*men) were included in the study. Endogenous hormone production was suppressed using GnRH agonists. Transdermal or oral Estradiol was administered to trans*women and transdermal or intramuscularly testosterone to trans*men.
Main results and the role of chance: The mean age at first visit was 20.9± 6,58 SD years in trans*men and 32.7 ± 15,90 SD years in trans*women. LH and FSH decreased in both sexes immediately after starting GnRHa, but not to levels below the detection limits. The sex steroids showed a rapid adaptation to the reference ranges of the desired sex. SHBG decreased significantly in trans*men, while hemoglobin increased significantly to the normal male ranges. There were no significant changes in BMI, but a trend towards weight gain in both sexes.
Limitations, reasons for caution: As the follow up period of 2 years is short, no long term safety assessments can be made so far.
Wider implications of the findings: CHT seemed safe in the study population. Since there were no significant differences and no pathological increase in liver enzymes, a routine check for liver enzymes does not appear to be necessary.
Trial registration number: Not applicable

P-575 Polycystic Ovarian Syndrome related hyperandrogenism and elite sport activity – a systematic review

C. Lamballk[1], E. Bos[1], M. Caanen[1]

[1]Amsterdam University Medical Centers Location VUMC, Reproductive Medicine, Amsterdam, The Netherlands

Study question: Is there a higher prevalence of PCOS among athletic women compared to the general population?
Summary answer: Based on the information of the current literature, no decisive evidence was found for a higher prevalence of PCOS among female elite athletes.
What is known already: The population of sporting women and physically active women at a high level of sport is increasing. This may lead to the Athlete Triad consisting of altered bone mineral density, low energy availability and menstrual dysfunction. It is suggested that Polycystic Ovarian Syndrome (PCOS) might be a cause for the menstrual disorders in female athletes. There is growing concern about androgen levels and top sports performance, doping control and fair play. The PCOS condition is positioned between two main issues in the field of female top sports as cause for menstrual irregularities related to higher endogenous testosterone levels.
Study design, size, duration: A systematic literature review was performed up to October 2019 in the following databases: PubMed, Cochrane, Embase and Sportdiscus. To be included in the review, papers had to include elite athletes women diagnosed with PCOS by the Rotterdam Criteria performing any type of sport at national or international level. Furthermore, the papers needed a focus on PCOS, hyperandrogenism, oligomenorrhea or amenorrhea prevalence outcomes and androgen measurement.
Participants/materials, setting, methods: Fourteen studies of an initial 536 were selected by two researchers independently. The included studies consisted of 10 observational cross-sectional studies, two case-control studies (observational and experimental) one prospective cohort and one retrospective cohort review of medical reports. The results are presented by outcome corresponding with the 3 hallmarks of PCOS and were plotted in forest plots.
Main results and the role of chance: PCOS and Hyperandrogenism showed a non significant and menstrual dysfunction a significant favoured prevalence in the athletes' group. The reported prevalence of PCOS diverged from 1.4% to 44%.
Limitations, reasons for caution: None of the studies primarily aimed at exploring the PCOS prevalence among elite athletes. Overall, the majority of the studies had a high risk of bias.
Wider implications of the findings: Exposure to higher androgen levels in female elite sport is currently a fierce matter of debate. This discussion requires higher quality data than currently available on prevalence of the most frequently occurring female natural condition of PCOS among top sport women. This merits well designed and powered prospective studies.
Trial registration number: not applicable

P-576 Cumulative Live birth rates in advanced maternal age (AMA) patients (40-43 years old) with autologous oocytes: the predictive factors

P. Oger[1], L. Delaroche[1], V. Barbet[2], X. Guettier[1], G. Kerbrat[1], V. Maget[1], G. Soudré[1], E. Genauzeau[1], P. Meicler[1], F. Lamazou[1]

[1]Ramsay santé Hôpital Privé de Parly 2, ART Center IFM P2, Le Chesnay, France ; [2]Capionis, biostatistics, Bordeaux, France

Study question: What are the predictive factors of the cumulative live birth rates (CLBR) in women aged between 40-43 years using their autologous oocytes?
Summary answer: In women aged between 40-43 years old, additional vitrified embryos is the only predictive factor to CLBR.
What is known already: In Vitro Fertilization (IVF) in AMA patients is becoming increasingly important. In this population, the low success rates and the high risks of miscarriages are mainly linked to the oocyte aneuploidy rates which increase with the maternal age. Several studies showed the probability of having a healthy baby in the AMA group of patients is less than 10% per cycle when using autologous oocytes and without any preimplantation genetic testing for aneuploidy (PGT-A). These low success rates question the management of AMA patients.
Study design, size, duration: This is a single-center retrospective study. The institutional review board approved this study and the patients'consent to the use of their data was prior obtained.
Participants/materials, setting, methods: All IVF cycles performed from November 2015 to December 2018 in women between 40-43 years old using their own oocytes were retrospectively analyzed. The main endpoint was the CLBR defined as a live-born delivery ≥28 weeks after the fresh or one of the subsequent frozen embryo transfers per pick-up. A multivariate logistic regression model was used to assess the influence of the clinically relevant factors. The p-values <0.05 were considered to be statistically significant.
Main results and the role of chance: A total of 569 IVF cycles in 316 couples were included. Mean female age was 41.4 ± 0.88 and mean AMH level was 2.0 ± 2.1 pg/mL. Using female age categories, the CLBR was 8.6% for 40-41 patients, 9.6% for 41-42 patients and 4.3% for 42-43 patients ; CLBR was improved in patients with supernumary vitrified embryos (OR [95%CI] = 2.78 [1.16-6.4] (p=0.02)). Multivariate logistic regression model did not highlight any other predictive factors on the CLBR: neither the maternal age (p=0.20), the etiology (p=0.80), the type of infertility (p=0.83), the smoking status (p=0.21), the AMH level (p=0.54) nor the number of mature oocytes (p=0.58) influenced individually the CLBR.
Limitations, reasons for caution: Although the main outcome was CLBR, the design was retrospective. The results of this study differed from those available in the current literature.
Wider implications of the findings: This study provides to AMA patients and clinicians a tool for building realistic expectations and providing accurate individual counselling. These preliminary results call for a prospective randomized controlled trial including higher number of cycles.
Trial registration number: IRB00010835

P-577 Late follicular phase ovarian stimulation protocol without exogenous pituitary modulators

Y. Fu[1], X. Zhu[1]

[1]Shanghai Ninth Hospital, Reproduction, Shanghai, China

Study question: Is it feasible to perform ovarian stimulation from late follicular phase without exogenous pituitary modulators during in-vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) treatments?
Summary answer: Late follicular phase ovarian stimulation could be performed without exogenous pituitary modulators.
What is known already: Gonadotropin-releasing hormone antagonist (GnRH-ant) was the most commonly-used modulator to prevent the premature luteinizing hormone (LH) surge when ovarian stimulation was initiated in the late follicular phase. Our team of researchers firstly introduced the usage of exogenous progestational agents as an alternative pituitary modulator to gonadotropin releasing hormone agonist (GnRH-a), and GnRH-ant for the prevention of premature LH surges in ovarian stimulation. Recently, we have documented

ovarian stimulation stared in late follicular phase with a dominant follicle diameter of ≥14mm before spontaneous ovulation could be performed without exogenous pituitary modulators.

Study design, size, duration: We retrospectively screened out the data of normal-ovulatory patients who performed ovarian stimulation in late-follicular phase with a dominant follicle diameter of ≥10 mm in the absence of exogenous pituitary modulators (namely late stimulation (LS) protocol) from women who treated with IVF/ICSI for the first time with the "freeze-all" strategy from May 2016 to December 2018, in our setting, and compared the reproductive results with early-follicular phase started ovarian stimulation using progesterone protocol.

Participants/materials, setting, methods: 404 participants were included: 116 subjects for the study group and 288 subjects for the control group. In the study group, only gonadotropin (Gn) was injected from the start of ovarian stimulation till the trigger day. In the control group, Gn and micronized progesterone soft capsule were added from menstrual cycle day 3. The primary outcome was the number of mature oocytes.

Main results and the role of chance: The number of mature oocytes was 9.67±5.33 in the study group and 9.38±5.15 in the control group (*P*=0.693). Total Gn dose (1962.28 ±517.06 versus 1626.04 ±311.75 IU) was greater and mean Gn duration (10.48±2.44 versus 8.78±1.53) was longer in the study group than in the control group (*P*<0.001). The number of follicles with diameter >14 mm was similar between the two groups, while number of follicles with diameter >10 mm in the study group was greater than that in the control group (13.33±6.67 versus 11.87±6.02, p=0.02). Additionally, there was no statistic difference in the number of mature oocytes between the two groups (9.67±5.33 in the study group versus 9.38±5.15 in the control group, P=0.693). Similarly, the two groups were comparable regarding number of oocytes retrieved, fertilized oocytes, cleaved embryos and good-quality embryos. No secondary premature LH surges were detected in all the participants. The clinical pregnancy rate was comparable in the two groups (54.55% versus 56.48%, P = 0.718). The implantation rate was similar in the two groups(36.94% vs. 37.77%, P=0.829). No differences were found in the rate of biochemical pregnancy, multiple pregnancy, ectopic pregnancy and early miscarriage (P > 0.05).

Limitations, reasons for caution: The limited cases and retrospective design resulted in the bias of our study. Further studies in a large sample size and continuous follow-up are still needed to determine the long-term safety of for children conceived with this novel protocol.

Wider implications of the findings: Our data suggest late follicular phase ovarian stimulation without exogenous pituitary modulators was a feasible protocol when performing random-start ovarian stimulation. In addition, it may be provided as an effective alternative for patients who choose frozen-embryo transfer (FET) during infertility treatment or fertility preservation.

Trial registration number: not applicable

P-578 Development of a new infertility treatment for resistant ovary syndrome through autoimmune suppression

Y. Tanaka[1], **K. Kawamura**[1]

[1]*International University of Health and Welfare school of medicine, Department of obstetrics and gynecology, Chiba, Japan*

Study question: Can suppression of autoimmune status using corticosteroid hormone induce follicle growth in patients with resistant ovary syndrome (ROS)?

Summary answer: Treatment of corticosteroid hormone, predonisolone (PSL) was effective for induction of follicle growth in ROS patients.

What is known already: ROS shows hyper gonadotropin and low estrogen, similar to premature ovarian insufficiency (POI). Infertility is the common complaint with ROS and POI. Although no follicle is found in ovaries of POI under ultrasound, ROS patients have normal number of antral follicles which are unresponsive to endogenous and exogenous FSH. Presence of genetic mutation or autoimmune antibodies to FSH or FSH receptor was found in some ROS patients, majority of causes are still unknown. So far only two case reports of live birth in ROS patients have been published by using an approach of in vitro maturation of oocytes .

Study design, size, duration: Case series study. From, April 2018 to Dec 2019, we enrolled four ROS patients with written informed consent after permission of this procedure from the ethical committee of the hospital.

Participants/materials, setting, methods: After diagnosis of ROS, the participants received 15-20mg/day PSL for 4weeks before starting ovarian stimulation. With continuous PSL treatment, we administrated estrogen plus progesterone for 10-14days to induce withdrawal bleeding. At initiation of bleeding, we started ovarian stimulation using 100mg clomiphene. The clomiphene treatment was continued up to 4weeks until their follicles reaching to 14-18mm in diameter. Then, 15,000-20,000IU hCG was injected. Oocyte retrieval was performed at 36hours after hCG.

Main results and the role of chance: The median age of participants was 37 years-old (range 26-41) with normal serum AMH (median 5.58 ng/ml, range 3.00-6.46) levels and normal antral count (range 7-15). However, they had increased serum FSH (median 44.6mIU/ml, range 19-95) and LH (median 31.4mIU/ml, range 8.2-51.4) levels as well as low estrogen levels (median 27.6 mIU/ml, range 0-52.6). Those patients showed no recent spontaneous follicle growth and long term amenorrhea (median 18.5 years, range 11-22 years). They also did not respond ovarian stimulation before this procedure. Among 4 patients, we succeeded follicle growth in two cases. In case 1, the follicle started to growth after 22−25 days of clomiphene treatment, whereas follicle growth could induced by15-19 days of clomiphene treatment in case 2. Total of 16 and 20 preovulatory follicles (range 3-5 and 2-7 per stimulation) were found and total 10 and 11 of mature oocytes were obtained in case 1 and 2, respectively. Seven out of 10 and 7 out of 11 oocytes were fertilized and all embryos developed to high-quality embryos (grade 1-3) based on Veeck criteria in case 1 and 2, respectively. All high-quality embryos were cryopreserved for future transfer under hormone replacement cycles.

Limitations, reasons for caution: The prevalence of ROS is extremely rare, the number of participants is small. We need to complete the procedure to check if the pregnancy and live birth will be achieved.

Wider implications of the findings: We developed a new approach for successful follicle growth to allow oocytes retrieval in ROS patients by autoimmune suppression using PSL. If we can detect the autoimmune antibodies responsible for ROS, this procedure could be a more useful method for groups of ROS patients hard to be treated.

Trial registration number: UMIN000034464

P-579 Peroxiredoxin 4 protects against ovarian ageing by ameliorating D-galactose induced oxidative damage in Mice

Y. Meng[1], **X.R. Liang**[1]

[1]*The First Affiliated Hospital of Nanjing Medical University, State Key Laboratory of Reproductive Medicine, The Center for Clinical Reproductive Medicine*

Study question: What the effect and underlying molecular mechanism dose Prdx4 protein play in D-galactose induced ovarian ageing?

Summary answer: D-galactose induced ovarian ageing is accelerated in Prdx4$^{-/-}$ mice, which results from accelerated apoptosis in granulosa cell via oxidative stress and ER stress-related pathways.

What is known already: Prdx4, a member of the Prdx family, is an important ER-resident antioxidant in cells. As revealed by our previous study, the expression of Prdx4 was detected in ovarian granulosa cells and closely related to ovarian function. We also discovered that it was located in ER of granular cell and involved in regulating the ER stress. D-galactose induced ovarian aging in mice has been extensively used to study mechanisms of diminished ovarian reserve (DOR).

Study design, size, duration: In this study, we established mice model with gene prdx4 knock out (Prdx4$^{-/-}$) by the CRISPR/Cas9 technology. Adult (5 months) wild-type and Prdx4$^{-/-}$ mice were both intraperitoneally injected with D-galactose (150 mg/kg/day) daily for 6 weeks. Ovarian function, oxidative damage, ER stress and granulosa cell apoptosis in the ovaries were evaluated in the two groups.

Participants/materials, setting, methods: We detected the representative estrous cyclicity during 12 consecutive days. Secondly, we calculated ovary-to-body weight ratio and detected the level of E_2. Thirdly, the number of different types of follicles were counted by hematoxylin and eosin (H&E) staining, and apoptosis in granulosa cells were tested by TUNEL. In addition, we observed the expression of senescence-associated protein and oxidative stress related factors via immunohistochemistry. Eventually, three ER stress pathways related markers were examined by western blot.

Main results and the role of chance: The HPG axis was more disrupted (*P*<0.01), and ovarian weight was relatively lower in the Prdx4$^{-/-}$ mice. The numbers of atretic follicles and apoptotic granulosa cells were significantly

increased in Prdx4$^{-/-}$ mice (P<0.05). In addition, the Prdx4$^{-/-}$ mice showed increased expression of oxidative damage-related factors (8-OhDG, 4-HNE, and NTY) and ovarian senescence-associated protein P16 (P<0.05). Moreover, levels of the proapoptotic factors CHOP and activated caspase-12 protein (P<0.01), which are involved in the endoplasmic reticulum stress pathway and level of cell apoptosis-related BAX protein were elevated in the ovaries of the Prdx4$^{-/-}$ mice.

Limitations, reasons for caution: This is a descriptive study in mice. More further experiments are necessary to explore the molecular mechanism for Prdx4 on ovarian function protection.

Wider implications of the findings: This study suggests that Prdx4 shows protective effect on ovarian function by reducing apoptosis of ovarian granulosa cell via downregulating oxidative stress and ER stress-related pathways.

Trial registration number: not applicable

P-580 GnRH agonist as an adjuvant to hCG for final oocyte maturation

I. Park[1], H. Sun[1], J. Kim[1], J. Lee[1], S. Shim[1], K. Lee[2], G. Jeon[3]

[1]Liora Fertility Center, Obstetrica & Gynecology, Busan, Korea- South ;
[2]Mamapapa& baby OBGY, Obstetrics and Gynecology, Ulsan, Korea- South ;
[3]Haeundae paik Hospital, Obstetrics and gynecology, Busan, Korea- South

Study question: Does triggering final oocyte maturation with standard dosage of hCG and additional GnRH agonist make better IVF outcomes?

Summary answer: GnRH agonist as an adjuvant to hCG for final oocyte maturation increases the number of mature oocytes and improves embryos quality.

What is known already: A single bolus of hCG has been the gold standard for triggering final oocyte maturation in ART cycles. GnRH agonist trigger has been utilized in GnRH-antagonist cycles for inducing final oocyte maturation while reducing the risk of ovarian hyperstimulation syndrome (OHSS). GnRH agonist trigger could help retrieving more mature oocyte. However, after GnRH agonist trigger, luteal phase defect results in lower pregnancy rate in fresh transfer cycle. Final oocyte maturation triggering with both standard dosage of hCG and GnRH agonist could help getting better IVF outcomes in GnRH antagonist cycles.

Study design, size, duration: This retrospective study included women undergoing IVF cycles using GnRH antagonist protocol between January 2013 and December 2017. A total of 4335 low and normo-responders were assessed.

Participants/materials, setting, methods: When at least 2 follicles reached 18 mm in diameter, final oocyte maturation was triggered by hCG 10000 IU alone or hCG 10000 IU plus GnRH agonist (leuprolide 1.0mg). And oocyte aspiration was done after 36 to 38 hours later. We divided the patients into two groups by triggering method. Group A (n=3776): trigger by hCG 10000 IU. Group B (n=559): trigger by hCG 10000 IU + GnRH agonist (leuprolide 1.0 mg)

Main results and the role of chance: In each group, age (36.3 vs. 36.4), endometrial thickness (10.1 mm vs. 9.9 mm) and the number of transferred embryos (1.9 vs. 1.9) did not show significant differences. There was no significant difference between two groups in total number of oocytes [9.9 vs. 10.4, P=0.054]. Oocyte recovery rate (total number of oocytes/ follicles>11mm) [118.7% vs. 131.0%, P<0.01], the number of mature oocytes [6.1 vs. 6.4, P=0.032] and the number of 2PN [6.7 vs. 7.1, P=0.029] were significantly higher in Group B than in Group A. Group B had higher embryo quality score [75.6 vs. 85.0, P<0.01] than Group A. Ongoing pregnancy rates were similar between two groups [43.9% (1535/3494) vs. 44.3% (203/458), P=0.190]. In both group, there was no case of severe OHSS.

Limitations, reasons for caution: This is retrospective study. The sample size of each group shows difference between two groups. Randomized control study will be needed to confirm our conclusions.

Wider implications of the findings: Triggering final oocyte maturation with standard dosage of hCG and additional GnRH-agonist increases the number of mature oocyte and 2PN. It also improves embryo quality. Routine use of dual trigger with hCG and GnRH agonist could be a good option to improve IVF outcomes without increasing OHSS or treatment burden.

Trial registration number: not applicable

P-581 Negative short-term effect of hysterectomy and bilateral salpingectomy on sex steroids, gonadotropins and AMH

J. Hutmacher[1], V. Uerlings[2], A. Günthert[3], J.M. Weiss[4]

[1]Cantonal Hospital of Frauenfeld, Obstetrics and Gynecology, Frauenfeld, Switzerland ;
[2]Cantonal Hospital of Lucerne, Obstetrics and Gynecology, Lucerne, Switzerland ;
[3]gyn-zentrum, Gynecology, Lucerne, Switzerland ;
[4]VivaNeo, Reproductive Medicine, Berlin, Germany

Study question: What is the course of sex steroids, gonadotropins and AMH after hysterectomy and bilateral salpingectomy?

Summary answer: DHEA-S, testosterone, androstenedione, estradiol, progesterone, LH, FSH, SHBG and AMH showed significantly decreased blood levels at the morning after surgery compared to preoperative/long-term samples

What is known already: Bilateral salpingectomy in benign conditions is widely performed to prevent ovarian cancer. Recent Meta-Analyses demonstrate no impairment of ovarian function of this procedure, even though an impaired postoperative ovarian stromal blood flow was described. Whether a hysterectomy and bilateral salpingectomy negatively affects sex steroids, gonadotropins and AMH remains to be determined.

Study design, size, duration: Prospective cohort study (February 2013 – January 2015) of 40 premenopausal women with ovary-sparing elective hysterectomy and bilateral salpingectomy for benign uterine conditions or premalignant high-grade cervical pathologies.

Participants/materials, setting, methods: Serum concentrations of DHEA-S, testosterone, androstenedione, estradiol, progesterone LH, FSH, SHBG, and AMH were assessed one day before, one day after and 4-6 weeks after surgery in a public hospital. Postoperative sampling was performed strictly at 07.30 a.m. (±1 hour).

Patients with presurgical evidence of malignancies, suspect ovarian morphology in transvaginal ultrasound, menopausal symptoms, menopausal hormone treatment, baseline FSH values >25 IU/L or baseline AMH values <0,6 pmol/l were excluded.

Main results and the role of chance: Mean age and BMI were: 44,83 years and 24,53 kg/m^2, respectively. At the day after surgery all hormones decreased significantly: DHEA-S ($p < 0.002$ mean: 4.10/2.37/3.69 mmol/L); testosterone ($p < 0.001$ mean: 0.95/0.49/0.86); androstenedione ($p < 0.24$ mean: 4.87/4.18/4.26 nmol/l); estradiol ($p < 0.05$ mean: 553.73/309.84/475.72 pmol/L); progesterone ($p < 0.13$ mean: 1.96/0.98/3.41 ng/ml); LH ($p < 0.3$ mean: 10.58/9.17/12.98 IU/L); FSH ($p < 0.46$ mean: 11.15/10.79/16.54 IU/L); SHBG ($p < 0.22$ mean: 74.64/67.13/73.65 nmol/L); and AMH ($p < 0.46$ mean: 5.23/4.99/7.12 pmol/l). There was no statistically significant correlation between hormone levels and operation time, blood loss or any further variable. Serum hormone changes did not correlate with age or BMI. However, by 4-6 weeks after surgery, circulating hormone values had returned to preoperative levels. We conclude that this hormonal decrease reflects an impaired hormonal regulation after surgery. These hormonal changes are transient, lasting no longer than 4-6 weeks.

Limitations, reasons for caution: These findings are based on a small sample size. The results need to be confirmed in larger patients groups and should include cortisol serum levels to depict stress levels.

Wider implications of the findings: These data reveal a postoperative dysregulation of the pituitary-gonad axis. It should be further explored how long these changes last and what kind of surgeries are involved. This might be important knowledge for the initiation of hormonal therapies after surgery.

Trial registration number: Switzerland: EKNZ

P-582 Recombinant-FSH or human-menopausal-gonadotrophin during ovarian stimulation resulted in similar mean euploid blastocyst rates per cohort of inseminated oocytes: an observational study on 961 patients.

S. Colamaria[1], D. Cimadomo[1], A. Vaiarelli[1], M. Giuliani[1], C. Argento[1], G. Bruno[1], S. Ferrero[1], G. Fabozzi[1], R. Mazzilli[1], A. Conforti[2], C. Alviggi[2], M. Schimberni[3], N. Ubaldi[4], L. Rienzi[1], F.M. Ubaldi[1]

[1]Clinica Valle Giulia, Genera centers for reproductive medicine, Rome, Italy ;
[2]University of Naples- Federico II, Department of Neuroscience- Reproductive Science and Odontostomatology, Naples, Italy ;
[3]Clinica Valle Giulia, Bioroma, Rome, Italy ;
[4]Catholic University of the Sacred Heart, Obstetrics and Gynecology, Rome, Italy

Study question: Do the type (recombinant-FSH or human-menopausal-gonadotrophin [hMG]) and dose of gonadotrophin adopted for controlled-ovarian-stimulation affect the euploid-blastocyst-rate per cohort of metaphase-II oocytes (m-EBR per MII)?
Summary answer: The type and total dose of gonadotrophins do not affect the m-EBR per MII.
What is known already: Controlled-ovarian-stimulation (COS) is one of the cornerstones of IVF. Its purpose is to obtain an adequate response in terms of oocytes' number and quality to improve treatments' efficacy and efficiency by obtaining several competent embryos. Although some data suggested that recombinant-FSH and hMG for COS in long agonist protocols perform similarly, the evidence is limited in antagonist protocols, i.e. the most commonly used at present. Therefore, the decision on which gonadotrophins should be used for COS is still uncertain, especially in patients at their first COS (naïve) and/or in freeze-all strategies.
Study design, size, duration: Observational study including all naïve patients (n=961, mean-maternal-age:39.2±3.5yr, mean-AMH:2.2±1.7ng/ml, mean-BMI:21.5±2.5) indicated for trophectoderm biopsy-based preimplantation-genetic-testing-for-aneuploidies (PGT-A) in the period 2013-2018. All patients underwent a GnRH-antagonist protocol. The kind of gonadotrophin was chosen depending on patients' compliance to the type of administration (pen for recombinant-FSH versus syringe for hMG) and gynecologists' judgment. The dose was established according to patients' characteristics. The primary outcome was the m-EBR per MII in the two groups.
Participants/materials, setting, methods: To achieve 80% power (α=0.05) to rule-out a 5%-difference in the primary outcome we required 792 and 158 naïve patients in the recombinant-FSH and hMG groups, respectively, assuming that 1/5 would use hMG. Only completed PGT-A cycles (live-birth achieved or no euploid embryos produced/left) with all MII-oocytes undergoing ICSI were included. Secondary outcomes were: mean number of oocytes, blastocysts and euploid blastocysts, maturation and blastocyst rates, and cumulative-live-birth-delivery-rate (CLBdR) per cycle.
Main results and the role of chance: 773(80.4%) and 188(19.6%) patients used recombinant-FSH and hMG, respectively. The groups were similar for maternal/paternal age, AMH, BMI, sperm factor, cause of infertility, and trigger of ovulation. The dose and duration of COS were lower with recombinant-FSH (2447±813IU versus 2672±871IU,p<0.01;9.7±1.7days versus 10.0±1.7day,p=0.02), nevertheless the number of cumulus-oocytes-complexes (COCs) was higher (n=8185,10.6±6.5 versus n=1709,9.1±5.7,p<0.01). The maturation rates per COCs (MII/COC) were similar (74%±19% versus 72%±21%,p=0.3), therefore also the number of MII was higher with recombinant-FSH (n=5827,7.5±4.4 versus n=1213,6.4±4.3,p<0.01). More blastocysts were biopsied with recombinant-FSH (n=1926,2.5±2.2 versus n=402,2.1±2.1,p=0.02), although the blastulation rate per MII was similar (33%±25% versus 33%±28%;p=0.5). The number of euploid blastocysts was slightly higher with recombinant-FSH (n=897,1.2±1.5 versus n=186, 1.0±1.5,p=0.05), while the m-EBR per blastocysts were comparable (34%±37% versus 30%±37%,p=0.1). Lastly, both the m-EBR per MII (15%±20% versus 14%±20%,p=0.14) and the CLBdR (n=251/773,32.5% versus n=50/188,26.6%;p=0.1) were similar. Once corrected for maternal age, AMH and sperm factor, neither the kind nor the dose of gonadotrophins was associated with the m-EBR per MII in a generalized-linear-model (p=0.2 and p=0.5). Once corrected for maternal age, number of oocytes and sperm factor, neither the kind nor the dose of gonadotrophins was associated with the CLBdR in a multivariate logistic-regression-analysis (p=0.2 and p=0.7).
Limitations, reasons for caution: The study was powered to exclude a 5%-difference in the primary outcome, therefore secondary outcomes are observational. A cost-effectiveness analysis is required. The choice of the gonadotrophin was based on patients' compliance with the administration method and gynecologist's judgment, thus randomized-controlled-trials investigating the CLBdR per cycle are still required.
Wider implications of the findings: Recombinant-FSH in naïve patients undergoing blastocyst culture, PGT-A and vitrified-warmed euploid embryo transfer(s) is suitable as first-line COS approach. Indeed, it may result in larger cohorts of oocytes of a similar competence as hMG, but involving a higher patients' compliance (lower dose, shorter COS and a well-accepted type of administration).
Trial registration number: None

P-583 GnRH-agonist and urinary-human-chorionic-gonadotrophin trigger of final oocytes' maturation result in similar oocytes' competence: an observational study on 2725 cycles with aneuploidy testing

C. Petriglia[1], D. Cimadomo[1], A. Vaiarelli[1], S. Colamaria[1], M. Giuliani[1], G. Bruno[1], R. Maggiulli[1], D. Soscia[1], M.G. Amendola[1], G. Nastri[1], F. Innocenti[1], G. Fabozzi[1], N. Ubaldi[1], F.M. Ubaldi[1], L. Rienzi[1]

[1]Clinica Valle Glulia, Genera center for reproductive medicine, Rome, Italy

Study question: Does the trigger of final oocytes' maturation (GnRH-agonist versus urinary-human-chorionic-gonadotrophin [u-hCG]) affect their competence during IVF cycles with preimplantation-genetic-testing-for-aneuploidies (PGT-A)?
Summary answer: GnRH-agonist and u-hCG involve similar maturation rates per oocyte-pick-up (OPU) and mean euploid blastocyst rates per cohort of metaphase-II oocytes (m-EBR per MII).
What is known already: The conventional trigger of final oocytes' maturation after controlled ovarian stimulation (COS) is hCG. Nevertheless, standing the growing application of GnRH antagonist protocols for COS, GnRH-agonist trigger has been introduced to almost eradicate the incidence of ovarian hyperstimulation syndrome (OHSS) in patients at high risk. While it is clear that this strategy affects the window of implantation when conventional luteal phase support is used, no evidence has been instead produced regarding its effect on oocytes' competence. Lastly, it is still uncertain whether GnRH-agonist trigger might be efficiently adopted in an unselected population of patients candidate to freeze-all.
Study design, size, duration: Observational study including 2725 OPU for PGT-A performed between 2013 and 2018 at a private IVF center (maternal age:39.5±3.3yr, AMH:2.1±1.8ng/ml, BMI:21.7±2.7). Also cycles without MII, zygotes or blastocysts were included. The primary outcome was the m-EBR per MII in the cycles adopting GnRH-agonist (n=1294) versus u-hCG (n=1431). The main secondary outcome was the mean maturation rate per OPU (MII/COCs). All outcomes were adjusted for patients'/cycles' putative confounders in generalized-linear-models and multivariate logistic-regression-analyses
Participants/materials, setting, methods: All patients had regular menstrual cycles. Different gonadotrophins and doses were used in antagonist COS protocols (recombinant-FSH,n=1048, 38.5%; recombinant-FSH+LH-activity,n=1065, 39.1%; hMG,n=282, 10.3%; biosimilar-FSH,n=90, 3.3%; corifollitropin-alpha+recombinant-FSH/hMG,n=240, 8.8%) according to patients' characteristics and gynaecologists' judgement. When ≥2 follicles reached a diameter ≥17–18mm, the trigger was performed 35hr before OPU with a subcutaneous bolus of GnRH-agonist (Buserelin:0.5ml) or intramuscular injection of u-hCG (10,000IU). Denudation, ICSI, blastocyst biopsy, comprehensive-chromosome-testing, and vitrified-warmed euploid blastocyst transfers were conducted after OPU.
Main results and the role of chance: The adoption of the GnRH-agonist trigger increased during the observational period (~10% to ~60%), especially in patients collecting >10 cumulus-oocyte-complexes (COCs) (~30% to ~80%). This reduced the incidence of patients referring symptoms of a moderate-severe OHSS from ~2% to none, with no impact on the competence of the retrieved oocytes. Overall, GnRH-agonist trigger was adopted in younger patients (38.8±3.4yr) who collected more COCs (n=17593, 13.6±7.4 per OPU) than u-hCG (40.2±3.1yr; n=10552 COCs, 7.4±4.6 per OPU). Nevertheless, the trigger adopted, also when adjusted per maternal age and number of COCs in a generalized-linear-model, did not associate with the mean maturation rate per OPU (p=0.1). A similar statistical approach outlined that the mean fertilization rate per MII, the mean blastulation rate per cohort of zygotes, and the mean-EBR per cohort of biopsied blastocysts were also independent of the trigger adopted (p=0.5, p=1.0, and p=0.3, respectively). Not even the primary outcome under investigation (i.e. m-EBR per MII) was affected by the trigger used (p=0.83). At last, the cumulative-live-birth-delivery-rate (CLBdR) per completed cycle (i.e. ≥1 live-birth achieved or no euploid embryos left/produced) was independent from the trigger adopted (Odds-Ratio: 1.0,95%CI 0.85-1.31, adjusted-p=0.6) in a multivariate-logistic-regression-analyses corrected for confounders.
Limitations, reasons for caution: This is a retrospective study. No cost-effectiveness analysis was conducted. GnRH-agonist trigger was performed independently from LH levels, although this might identify specific subpopulations of patients with a suboptimal response. Lastly, properly-conducted

Randomized-Controlled-Trials are still eagerly requested, especially in antagonist COS protocols.
Wider implications of the findings: Throughout the years the adoption of GnRH-agonist trigger after antagonist COS protocols has sharply increased for cycles planned for PGT-A with blastocyst culture, biopsy, and freeze-all. This did not impact oocytes' competence, while almost eradicating OHSS. U-hCG trigger is still used in ~50% of the patients collecting ≤10 COCs.
Trial registration number: None

P-584 Role of Homeostasis model assessment (HOMA2-IR), Quantitative insulin sensitivity check index (QUICKI) and Irisin in diagnosing insulin resistance among young girls with Polycystic ovarian syndrome

H. Sagili[1], B. Sharan[1]

[1]JIPMER, Obstetrics and Gynaecology, Puducherry, India

Study question: Do HOMA2-IR, QUICKI and Irisin diagnose additional cases of insulin resistance in young girls with Polycystic ovarian syndrome (PCOS) in comparison with 75 gm OGTT?
Summary answer: HOMA2-IR, QUICKI and Irisin appear to detect additional cases of insulin resistance in young girls with PCOS in comparison with 75 gm OGTT.
What is known already: Young girls with PCOS have a predilection to become insulin resistant with an increased risk of impaired glucose tolerance (IGT) and type 2 Diabetes Mellitus (DM). Because impaired glucose tolerance is often asymptomatic, screening for insulin resistance (IR) is recommended. The gold standard method of assessing Insulin resistance namely Euglycemic hyper-Insulinemic clamp technique is expensive and not practical. Currently 75 gm oral Glucose tolerance test (OGTT) is being offered as a screening method for insulin resistance but might not identify all cases of IR. The role of other surrogate markers for diagnosis of IR is not yet established.
Study design, size, duration: This cross sectional study was carried out over a period of two years.82 young girls aged 15-19 years -41 young girls with PCOS were compared to 41 age matched controls who had presented with minor gynaecological complaints such as dysmenorrhea, white discharge per vaginum, lower abdominal pain etc. Excusion criteria were thyroid dysfunction,hyperprolactinaemia and treatment with Insulin sensitizers. Consecutive sampling was carried out.
Participants/materials, setting, methods: Girls recruited from Gynaecology outpatient department of a tertiary care hospital underwent testing for Fasting- Serum Irisin, Insulin; Blood Glucose and second hour Serum Insulin; Blood glucose after 75 gm glucose. HOMA 2-IR and QUICKI were calculated using computer software and Serum Irisin was measured by ELISA. Cut offs for HOMA2-IR, QUICKI and Irisin were defined based on population based studies as >2.5, 0.383+0.007 for nonobese / 0.331+ 0.010 for obese and 15.43 ng/ml respectively.
Main results and the role of chance: Almost half the cases were obese, another 22% were overweight in our study and the body mass index between cases and controls was statistically significant (p<0.0001).78% of the cases had all three features of PCOS according to Rotterdam's criteria. Infrequent menstrual cycles and clinical and/or biochemical signs of hyperandrogenism were seen in 14.7% and infrequent cycles and polycystic ovaries were present in 7.3%. 51% of cases had impaired glucose tolerance on 75 gm OGTT and there were no cases of overt diabetes. 43.9% cases had IR based on HOMA2-IR compared to 21% controls (p=0.03). 34% cases compared to 17% controls had IR based on QUICKI (p=0.038).19% cases compared to 9.7% controls had IR based on fasting serum Irisin (p=0.211). Overall 21 cases were diagnosed to be insulin resistant based on 75 gm OGTT ; 18 additional cases (43.9%) were diagnosed as insulin resistant based on a panel of markers (HOMA2-IR ,QUICKI and fasting serum Irisin). 11 and 8 cases (26.8% and 19.5%) were diagnosed based on only high HOMA2-IR and QUICKI values respectively. Similarly, 3 cases (7.3%) were diagnosed as insulin resistant based on only high fasting serum Irisin values .
Limitations, reasons for caution: The study was done on a small sample and as a case-control study for the findings to be applicable to a larger population. Further, follow up of these subjects may yield additional insight into the progression of insulin resistance and type 2 DM in young girls with PCOS.
Wider implications of the findings: As young girls with PCOS are at high risk of developing IGT, early identification of IR by a panel of HOMA2-IR, QUICKI and Irisin in addition to GTT may play a pivotal role in institution of lifestyle changes that may help in delaying the progression to type 2 DM.
Trial registration number: not applicable

P-585 Prokineticin receptor 2 (PROKR2) mutations in functional hypothalamic amenorrhoea

A. Tranoulis[1], D. Mavrogianni[2], A. Soldatou[3], A. Pampanos[2], T. Leoutsakou[4], P. Drakakis[5], D. Loutradis[6], L. Michala[7]

[1]Guy's and St Thmas' NHS Foundation Trust- King's College, Obstetrics and Gynaecology, London, United Kingdom ;
[2]Alexandra Hospital- Athens University Medical School, Molecular Biology Unit-Division of Human Reproduction, Athens, Greece ;
[3]'P&A Kyriakou' Children's Hospital- National and Kapodistrian University of Athens- School of Medicine, Second Department of Paediatrics, Athens, Greece ;
[4]Alexandra Hospital- National and Kapodistrian University of Athens, Department of Molecular Biology, Athens, Greece ;
[5]Alexandra Hospital-Athens University Medical School, Division of Human Reproduction- IVF Unit- First Department of Obstetrics and Gynaecology, Athens, Greece ;
[6]Alexandra Hospital- Athens University Medical School, Division of Human Reproduction- IVF Unit- First Depertment of Obstetrics and Gynaecology, Athens, Greece ;
[7]Alexandra Hospital- National and Kapodistrian University of Athens- School of Medicine, First Deparment of Obstetrics and Gynaecology, Athens, Greece

Study question: To identify any possible mutations in prokineticin recptor 2 (PROKR2) gene in subjects with functional hypothalamic amenorrhoea (FHA).
Summary answer: PROKR2 mutation screening in 41 women with FHA resulted in the identification of eight novel different mutations in five patients.
What is known already: Although the proximate cause of FHA is the abnormal gonadotropic-releasing hormone (GnRH) secretion, it is seemingly more than an isolated desynchronisation of the GnRH axis. There is fair evidence to support the association of FHA with behavioural, emotional, cognitive and psychological factors. However, it remains uncertain, whether this variability is also attributable to a genetic predisposition. The PROK2 and PROKR2 singling pathway is implicated in the cause of idiopatic hypogonadotropic hypogonadism (IHH) and Kallmann's syndrome. Two heterozygous PROKR2 mutations (c.254 G>A and c.518 T>G) have also been identified amongst patients with FHA. The latter were found to be loss-of-function mutations.
Study design, size, duration: This is a prospective, single-centre study carried out at a tertiary referral clinic for gynaecological endocrinology and paediatric & adolescent gynaecology, spanning the period January 2016 to June 2019. We analysed the coding sequence of PROKR2 in 84 women: 41 with FHA, 23 with IHH, and 20 healthy controls respectively.
Participants/materials, setting, methods: Genomic DNA was extracted from peripheral blood samples using PureLink Genomic DNA Kits for purification of genomic DNA. PCR was performed using primers designed to amplify the two coding exons and the exon-intron boundaries. The amplified PCR products were sequenced using the ABI Prism 3130 Genetic Analyzer, Applied Biosystems. Variants were validated using the sequencing Analysis v 5.2 software.
Main results and the role of chance: Eight novel PROKR2 mutations were identified in five of the 41 women with FHA, either in heterozygous state (two cases) or in homozygous or compound heterozygous state (three cases)**:** 241 c.G>C, c.296 G>T, c.375 A>G, c.404 A>G, c.404 C>G, c.410 C>A, c.421 G>A, and c.475 G>T. Furthermore, three novel PROKR2 heterozygous mutations were identified in three of 23 women with IHH: c.246 C>T, c.252 C>T and c.259 G>C. Reproductive phenotypes ranged from absent to partial puberty to complete reversal of gonadotropin-releasing hormone deficiency following discontinuation of treatment.No mutations were found in the cohort of 20 controls with normal menstrual cycles.
Limitations, reasons for caution: Further research to ascertain the functional impact of these variants is warranted. Differences in the genetic backgrounds of various ethnic populations should also be considered.
Wider implications of the findings: Identification of gene mutations underlying FHA may lead to new pathophysiology insights, improved diagnostics, and novel treatment approaches.
Trial registration number: not applicable

P-586 Impact of gonadotropin genetics profile and ovarian reserve on controlled ovarian stimulation outcomes

A. Rebecchi[1], E. Papaleo[1], M. Mignini Renzini[2], C. Alviggi[3], A. Marino, Alessandro[4], C. Brigante[5], A. Conforti[3], M. Molgora[1], R. Iemmello[6], I. Carbone[7], L. Quaranta[1], A. Allegra[4]

[1]San Raffaele Hospital, Obstetrics and Gynecology, Milan, Italy ;
[2]Biogenesi Reproductive Medicine Center - Istituti Clinici Zucchi Monza, obstetric and gynecology, Monza, Italy ;
[3]University of Naples- Federico II, Department of Neuroscience- Reproductive Science and Odontostomatology, Naples, Italy ;
[4]ANDROS Day Surgery Clinic, Reproductive Medicine Unit, Palermo, Italy ;
[5]Biogenesi Reproductive Medicine Centre- Istituti Clinici Zucchi, Reproductive Medicine, Monza, Italy ;
[6]Ospedale Maggiore Policlinico- Mangiagalli and Regina Elena, Infertility Unit, Milan, Italy ;
[7]Ospedale Maggiore Policlinico- Mangiagalli and Regina Elena, Obstetrics and Gynecology, Milan, Italy

Study question: o assess the difference between expected, through standardized-3D AFC, and retrieved oocytes in relation to gonadotropin genetic profile after standard COS with 150 UI/day r-FSH.
Summary answer: In good prognosis women, specific genetic variants seem to partially influence ovarian stimulation, despite no relevant impact on pregnancy rates was observed.
What is known already: Among fertility treatment, controlled ovarian stimulation represents the key step, with the main aim to obtain a certain number of competent oocytes that will enable the best probability of achieving a live birth. The ovarian response to FSH in individuals is difficult to predict. Indeed, women with similar demographic, anthropometric and gonadotropin profiles may have different response to r-FSH due to the "sensitivity" of follicles to exogenous FSH itself. Several studies demonstrated that such "sensitivity" could be influenced by specific genotype characteristics. However, the evidence in this sense seems to be controversial and inconclusive.
Study design, size, duration: We performed a multicenter, longitudinal, prospective, interventional, cohort pilot-study, enrolling 119 women attending four clinical Centers of medically assisted reproduction from August 2016 to November 2018.
Participants/materials, setting, methods: Infertile normo-gonadotropin patients, aged between 34 and 39, at their first COS, with normal ovarian reserve (AFC between 8 and 16) measured with 3D automated ultrasonography. Blood samples were collected for gonadotropin genetic polymorphisms assessment and hormone assay. Stimulation protocol consisted of standard 150 IU/day r-FSH for the whole stimulation period starting from basal assessment, GnRH antagonist starting from day 5-6 of stimulation, and trigger when at least two follicles reached >16 mm diameter.
Main results and the role of chance: 106 patients completed the stimulation protocol. The mean basal 3D follicular count was 13.4, the mean of follicles > 16 mm at induction was 5.8 and the mean number of retrieved oocytes was 8.4. No significant correlation was found between the number of retrieved oocytes and different genotypes for each polymorphism. However, after 5 days of stimulation, we observed a significant impact of AG genotype for FSHR p.N0680S on the percentage of observed follicles ≥10 mm compared with genotypes AA (p = 0.04) and GG (p = 0.005). Nonetheless, at day of trigger, no significant differences were found in the number of follicles ≥16 mm. This may be due to a fastest development of follicles in the first part of stimulation for women carrying AG genotype. LHCGR2 GG genotype leads to a shorter stimulation compared with genotypes AA (p = 0.008) and GA (p = 0.006). The same result was obtained for homozygous GG genotype for the FSHB-211 polymorphism compared to the genotype GT (p = 0.04). Significant correlation was found between BMI and heterozygous genotype of the FSHR p.N0680S (OR= 1.28 [1.09-1.52] p=0.004). We obtained 36.8% of ongoing pregnancies per started cycle.
Limitations, reasons for caution: Strict inclusion criteria limited the enrolment of subjects planned before the study. Thus, the main findings of our study could be underpowered. However, this is the first prospective study in which polymorphisms were associated to AFC assessed by a reliable, standardized automated method together with centralized AMH assessment.
Wider implications of the findings: In normo-responder patients, the impact of pharmacogenomics in the personalization of treatment protocol is far from clearly correlating with IVF-ICSI pregnancies. On the other hand, a marginal effect on ovarian stimulation and anthropometric characteristics of women seems to emerge. Further biggest studies are needed.
Trial registration number: not applicable

P-587 Body Mass Index stratified Anti-Müllerian hormone thresholds for diagnosing Polycystic Ovary Syndrome

X. Liu[1], Y. Guo[1]

[1]The First Affiliated Hospital of Zhengzhou University, Reproductive Medicine Center, Zhengzhou, China

Study question: Should Anti-Müllerian hormone (AMH) threshold for diagnosing Polycystic Ovary Syndrome (PCOS) be stratified according to different Body Mass Index (BMI)?
Summary answer: PCOS patients with lower BMI had higher serum AMH levels and AMHR mRNA expression in granulosa cells and we proposed BMI stratified AMH thresholds.
What is known already: Serum AMH levels are higher among patients with PCOS than healthy women and PCOM (Polycystic Ovary Morphology) women with normal ovulation. While AMH has been reported to be positively or non-significantly related to BMI in infertile women without PCOS, in women with PCOS, AMH appears to be negatively correlated with BMI levels. AMH is believed to be a surrogate indicator of the antral follicle count (AFC) for diagnosing PCOS. However, the heterogeneity of PCOS patients has seldom been considered when calculating cut-off values.
Study design, size, duration: This was a retrospective cohort study based on 4347 infertile women under 35 years old treated in a reproductive medicine centre of a university-affiliated hospital from February 2016 to May 2018. Granulosa cells(GCs) were collected from 22 patients undergoing transvaginal oocyte retrieval after controlled ovarian hyperstimulation with agonist protocol from December 2019 to January 2020.
Participants/materials, setting, methods: The study population included 1220 patients with PCOS, 1386 patients with PCOM and 1741 normal controls. We divided them into normal-weight groups and overweight groups separately based on a BMI threshold of 24 kg/m^2 according to a guideline based on the Chinese population. RNA samples were extracted from GCs from 13 PCOS patients and 9 normal controls. Expression of AMHR mRNA were evaluated by RT-qPCR.
Main results and the role of chance: Regardless of normal weight or overweight, PCOS patients tend to have higher AMH levels (P<0.001) and slightly higher AMHR expression in GCs (Normal weight: 2.69±2.59 vs 1.04±1.01, P=0.088; Overweight: 1.78±1.22 vs 0.82±0.42, P=0.116) than control groups. In the PCOS group, normal-weight women had higher serum AMH levels than overweight women (P<0.001) and slightly higher AMHR expression(P=0.133,0.180, respectively), while in the control groups, there was no such difference. The cut-off level for AMH for diagnosing PCOS was 4.77 ng/ml in the normal weight group and 4.15 ng/ml in the overweight group with sensitivity of 81.8% and 85.8%, specificity of 90.5% and 84.2%, respectively.
Limitations, reasons for caution: The current GC samples collected from patients were limited and the expression pattern of AMHR may be affected by the medication used during COH, thus, further experiments including larger sample size and validation with the animal model are needed.
Wider implications of the findings: This study agreed with the former study that AMH levels of patients with PCOS were affected by their BMI. Our study suggested that BMI should be taken into consideration when assessing endocrine indicators of women with PCOS, such as AMH, in both clinical practice and basic research.
Trial registration number: 81571409

P-588 The activated mTOR-S6K signaling pathway increases DNA damage in follicles of women with polycystic ovary syndrome

G. Jing[1], S. Cai[2], G. Lin[3]

[1]Reproductive and Genetic Hospital of CITIC-Xiangya, Reproduction Medicine Center, Changsha, China ;
[2]Reproductive and Genetic Hospital of CITIC-Xiangya, Reproductive Medicine Center, Changsha, China ;
[3]Central South University, School of Basic Medical Science, Changsha, China

Study question: Does the follicular microenvironment of polycystic ovary syndrome (PCOS) change by regulating the expression of mammalian rapamycin (mTOR) pathway targets?
Summary answer: The mTOR-S6K signaling, by controlling DNA damage response and genome stability of granulosa cells and oocytes, maintains follicular microenvironment of female ovarian.
What is known already: Recent researches suggest that mTOR pathway, which was found to be especially active in PCOS, is considered to have close relationship with the pathogenesis of PCOS. In previous studies, we determined the role of mTOR by use of conditional knockout mice model. mTOR-dependent pathways in primordial or growing oocytes differentially affected downstream processes including follicular development, sex-specific identity of early granulosa cells, maintenance of oocyte genome integrity, oocyte gene expression, meiosis, and preimplantation developmental competence. However, the underlying mechanism between mTOR pathway and pathophysiological changes of PCOS remains largely undetermined.
Study design, size, duration: In this study, patients with PCOS (n = 4) diagnosed according to the 2003 Rotterdam standard underwent partial or wedge resection of the ovary. The ovarian tissues used as control were obtained from female (n=3) who underwent ovariectomy for fertility preservation. Samples were fixed and analysed by immunohistochemistry to determine mTOR pathway activation and expression of markers reflecting DNA damage, cell apoptosis and proliferation. Mtor-conditional knockout mice was performed to examine the underlying molecular mechanism.
Participants/materials, setting, methods: Ovarian tissues of PCOS patients and Mtor- GcKO mice were used as study models. Ovarian tissues were fixed and analysed histologically. Immunostaining for: 1) the expression of MTOR and the activated form of its major downstream effectors—phosphorylated RPS6KB1, phosphorylated ribosomal protein S6 (pRPS6), phosphorylated EIF4EBP1. 2) γH2AX as a marker of DNA damage; 3) CASPASE3 as a marker of cells apoptosis; 4) KI67 as a marker of cells proliferation;
Main results and the role of chance: We found that nuclear expression of p-RPS6 level was increased in oocytes of primary and secondary follicles in PCOS patients compared with control group. In the PCOS group, γH2AX expression in oocytes and its surrounding granulosa cells of non-growing and primary follicles significantly increased ($p<0.05$). Expression of KI67 in granulosa cells of growing follicles showed no significant differences between any groups ($p>0.05$). The results of immunohistochemistry analysis showed that the levels of the active forms of phosphorylated MTOR (p-MTOR) and its downstream effectors were regularly expressed in oocytes and granulosa cells at different stages of folliculogenesis in mice. A major downstream effector of MTOR activation, p-RPS6, was gradually increased in different stages of oocyte as follicle development and strongly expressed in cumulus cells and pre-antral granulosa cells but barely detectable in mural granulosa cells. The results of Western blot showed that BMP15 was significantly decreased in oocytes collected from secondary follicles in Mtor- GcKO mice. Immunofluorescence staining of γH2AX revealed more DNA double-strand breaks (DSBs) in Mtor-GcKO oocytes of the early- stage growing follicles.
Limitations, reasons for caution: Due to the limitation by PCOS female tissue acquisition, the ovarian tissue detected in this study was obtained from the patients who need to perform ovarian wedge resection. Additionally, the underlying molecular link between mTOR pathway and DNA damage repair should be further evaluated.
Wider implications of the findings: These results reveal an important function of the mTOR-S6K signaling in DNA damage response and suggest a mechanism related to genome stability in the follicular microenvironment of PCOS patients. Our findings may subsequently provide valuable resources for developing targeted interventions aimed at improving follicle recruitment in vivo for PCOS patients.
Trial registration number: NA

P-589 True Natural Cycle outweighs Hormone Replacement Therapy in euploid frozen embryo transfer

A. Bayram[1], N. De Munck[1], I. El Khatib[1], A. Arnanz[1], A. Abdala[1], A. El Damen[1], L. Melado[1], B. Lawrenz[1], H. Fatemi[1]

[1]IVIRMA Middle East Fertility Clinic LLC, IVF Lab, Abu Dhabi, United Arab Emirates

Study question: Does the cycle regimen used for endometrial preparation affects the outcomes in euploid frozen embryo transfer (FET) cycles?
Summary answer: Clinical pregnancy and implantation rates were significantly increased in natural cycle (NC) as compared to hormone replacement therapy (HRT).
What is known already: The gold standard protocols in FET cycles are HRT, true or modified NC. When comparing live birth and clinical pregnancy rates (CPR), the superiority of one regimen over another has not been demonstrated. NC can only be performed in women with regular ovulatory menstrual cycles, while HRT cycles can be used in women with regular and irregular menstrual cycles. An increased early pregnancy loss has been reported in previous studies in HRT compared to NC, which could be related to the higher proportion of patients with polycystic ovary syndrome (PCOS) or the presence of bias linked to possible embryo aneuploidy.
Study design, size, duration: This single center retrospective cohort study included a total of 802 single/double euploid FET cycles between March 2017 and October 2019. Trophectoderm biopsy samples were subjected to Next Generation Sequencing to test the ploidy state. Vitrification and warming were performed using the Cryotop method (Kitazato, Biopharma). The CPR, implantation rate (IR) and early pregnancy loss (EPL) were evaluated in euploid frozen embryo transfers between true NC and HRT.
Participants/materials, setting, methods: The following patient characteristics were analyzed: age, Anti Müllerian hormone (AMH), body mass index (BMI), primary or secondary infertility, duration of infertility, uterine abnormalities (diagnosed by ultrasound), endometrial thickness, quality of the transferred embryo/s, difficulty of the transfer (requirement of additional instrumentation), day of biopsy and single (SET) or double (DET) embryo transfer. PCOS patients were excluded. The primary aim was to evaluate whether the clinical pregnancy and implantation were affected by the cycle regimen.
Main results and the role of chance: The mean patient age was 34.02±5.10 vs 33.34±5.52 years in NC and HRT. The FET was performed in a NC (n=294, 36.6%) or HRT cycle (n=508, 63.4%). Clinical pregnancy rate was significantly higher in NC; 66.3% vs 57.1% in HRT ($p=0.029$) while EPL rate was significantly lower in NC; 1.26% vs 5.05% ($p=0.025$), respectively. Also, the implantation rate was significantly higher in NC as compared to HRT (64.6%±46.81 vs 57.6%±47.93; $p=0.046$).
Patient characteristics (age, AMH, BMI, duration of infertility, uterine abnormalities, endometrial thickness, embryo quality, embryo transfer difficulty, biopsy day and SET/DET) were similar between both groups compared.

Multivariate logistic regression model was performed with all significant values of the univariate model ($p<0.20$), to check the factors predicting the clinical pregnancy outcome. Following characteristics showed a significant ($p<0.05$) effect on CPR: AMH: OR: 0.889 [0.795-0.995], $p=0.039$, age: OR: 1.36 [1.001-1.071], $p=0.04$, transfer of a top-quality blastocyst: OR: 7.445 [3.552-15.604], $p=0.001$, ET difficulty [Difficult vs Easy]: OR: 0.575 [0.347-0.954], $p=0.032$ and cycle regimen [NC vs HRT]: OR: 1.482 [1.035-2.121], $p=0.031$.
Limitations, reasons for caution: A prospective randomized study with large sample size would have minimized potential limitations as compared to this retrospective design.
Wider implications of the findings: Patients with regular menstrual cycles should be offered a natural FET cycle to achieve better outcomes in terms of clinical pregnancy and implantation rates.
Trial registration number: NA

P-590 Should we measure serum LH levels at the initiation of ovarian stimulation in a GnRH antagonist downregulated IVF/ICSI cycle?

F. Di Guardo[1], A. Racca[1], B. Popovic-Todorovic[1], S. De Rijdt[1], G. Verheyen[1], H. Tournaye[1], M. De Vos[1], C. Blockeel[1]

[1]Universitair Ziekenhuis Brussel- Vrije Universiteit Brussel, Centre for Reproductive Medicine, Brussels, Belgium

Study question: Does the serum LH level at the onset of ovarian stimulation (OS) in a GnRH antagonist cycle have an impact on reproductive outcomes?
Summary answer: The serum LH level at the start of GnRH antagonist cycles has no impact on live birth rates (LBRs) nor cumulative live birth rates (CLBRs).
What is known already: Previous studies suggested that elevated LH levels in the early follicular phase could be associated with lower pregnancy rate. Introducing a GnRH antagonist from Day1 onwards instead of Day 6, has been shown to suppress high LH levels at the start of OS for intrauterine insemination

in polycystic ovarian syndrome (PCOS) patients. A comparison between the administration of GnRH antagonist on Day 1 versus Day 6 of stimulation in regular IVF cycles has also been performed, which demonstrated no significant differences neither in follicular development nor in the oocytes maturity, however, so far, the effect on reproductive outcomes remains unknown.
Study design, size, duration: This was a retrospective, single-centre cohort study including all women (aged 18-43 years) undergoing their first ovarian stimulation for IVF/ICSI in a tertiary referral university hospital between 2009 and 2018. A total of 8329 hCG-triggered GnRH antagonist cycles were included. Cycles with hormonal pre-treatment, IVM, managed natural cycles, oocyte donation, and PGT were excluded from the analysis.
Participants/materials, setting, methods: LH serum levels at the initiation of the cycle were studied in all included patients. Cycles were divided into two groups based on a LH threshold level of 10 UI/L. The LH grouping was repeated using a LH threshold level of 15 UI/L in order to obtain cycles with very-high basal LH level. The primary outcome was CLBR. The secondary outcome was LBR.
Main results and the role of chance: General population baseline characteristics were average female age (33.6 ± 5 years), average basal LH (5.6 ± 3.1 UI/L) and mean number of oocytes retrieved (9 ± 6.1). The population was divided into a group of 7758 cycles with basal LH<10 IU/L and a group of 571 cycles with basal LH ≥10 IU/L. The multivariate analysis adjusting for potential confounders did not show any difference between the two groups in terms of CLBR (31.8% vs. 30.9%, p value=0.678) nor LBR (23.1% vs. 21.4%, p value=0.339). The analysis was repeated with the basal LH threshold level set at 15 IU/L, resulting in a group of 8216 cycles (LH<15 IU/L) and 113 cycles (LH ≥ 15 IU/L). Although LBR was comparable (23% vs. 23%, p value=0.997), CLBR in cycles with basal LH ≥ 15 IU/L was significantly higher compared to those in cycles with LH<15 IU/L (31,7% vs. 41,7%, p=0.05). Multivariable regression analysis showed that lower age, a diagnosis of Rotterdam PCOS and higher number of cryopreserved embryos were significantly associated with CLBR (adjusted OR = 0.94, 95% CI=0.92-0.94, p value <0.001; 1.25, 95% CI=1.08-1.46, p value=0.02; 1.29, 95% CI=1.26-1.32, p value <0.001 respectively).
Limitations, reasons for caution: In spite of the large dataset and the rigorous methodological approach, the presence of biases related to the retrospective design of the study cannot be excluded.
Wider implications of the findings: Based on our data, the assessment of basal LH levels at the initiation of an hCG-triggered GnRH antagonist cycle could be omitted.
Trial registration number: not applicable

P-591 The relation of eating disorder and polycystic ovarian syndrome in female

M. Bazrafkan[1], M. Amiri[2], A. Nadjarzadeh[3]

[1]Reproductive Biotechnology Research Center- ACECR, Avicenna Research Institute, tehran, Iran ;
[2]Shahid Sadoughi University of Medical Sciences- Faculty of Health, Nutrition, Yazd, Iran ;
[3]Shahid Sadoughi University of Medical Sciences-Faculty of Health, Nutrition, Yazd, Iran

Study question: The aim of this study was to clarify the prevalence of eating disorder and relation with BMI and quality of life in women with PCOS.
Summary answer: Women with PCOS had a higher BMI and eating disorder. Quality of life scores decrease by the eating disorder and body mass index increase.
What is known already: Polycystic ovary syndrome(PCOS) is an endocrine disorder in women, that can lead to infertility. Eating disorder has been suggested as one of the leading causes of obesity that is a main risk factor to PCOS. Also, women with PCOS had lower quality of life due to obesity and overweight that are the largest contributor to poor quality of life. On the other hand, obesity and weight loss is major concern in this women that lead to depression and lower quality of life.
Study design, size, duration: This cross sectional study was conducted in Avicenna Research Institute by 150 participants with polycystic ovary syndrome. The data collection was performed by using of the three structural questionnaire including demographic questionnaire, Eating Disorder Questioner(EDE-Q) and Health-Related Quality of Life Questionnaire (PCOSQ).
Participants/materials, setting, methods: 150 participants with polycystic ovary syndrome conducted in this study,The data collection was performed by using of the three structural questionnaire including demographic questionnaire, Eating Disorder Questioner(EDE-Q) and Health-Related Quality of Life Questionnaire (PCOSQ).
Main results and the role of chance: Prevalence of eating disorder was 26.3%. Body mass index(BMI) was higher in women with eating disorder(P=0.001). Also results showed significant correlation between EDE-Q score and BMI(P=0.000). Regarding to the relationship between EDE-Q score and quality of life, the results indicated that the quality of life decreased as the EDE-Q score increased(P=0.000). On the other hand, BMI and quality of life were also inversely correlated(P=.033).
Limitations, reasons for caution: no limitation
Wider implications of the findings: Clinicians should be aware of diagnosis and management of eating disorder in infertile women specially PCOS patients to prevent its complications.
Trial registration number: IR.SSU.SPH.REC.1397.149

P-592 Ovulatory women with polycystic ovaries and their clinical outcome following individualised ovarian stimulation based on AMH and body weight.

H. Visnova[1], E. Papaleo[2], F. Sánchez Martin[3], K. Koziol[4], B. Klein[5], B. Mannaerts[6]

[1]IVF CUBE, Reproductive Medicine, Prague 6, Czech Republic ;
[2]San Raffaele hospital, Obstetrics and Gynaecology Dept, Milan, Italy ;
[3]Ginemed, Fertility Clinic, Sevilla, Spain ;
[4]Novum Warsaw, Fertility Clinic, Warsaw, Poland ;
[5]Ferring Pharmaceuticals, Global Biometrics, Copenhagen, Denmark ;
[6]Ferring Pharmaceuticals, Reproductive Medicine and Maternal Health, Copenhagen, Denmark

Study question: How does the efficacy and safety of individualised fixed dosing regimen compare to conventional dosing for ovarian stimulation in patients with polycystic ovaries?
Summary answer: The ongoing pregnancy rates were similar but the incidence of early moderate/severe OHSS and/or preventive interventions for OHSS was three times lower following individualised stimulation.
What is known already: Individualised dosing of follitropin delta targets a mean of 11 oocytes (range 8-14 oocytes) and has proven to optimize ovarian response resulting in similar efficacy and improved safety compared to conventional ovarian stimulation. The safety improvement is positively associated with serum AMH levels, which is a biomarker of polycystic ovarian morphology.
Study design, size, duration: Retrospective comparative analysis of a subset of 153 women with polycystic ovaries with AMH > 35 pmol/l. Following ovarian stimulation, women with <25 follicles ≥12 mm were triggered using recombinant hCG and were eligible for fresh blastocyst transfer. Women with ≥25 follicles ≥12 mm were either triggered with GnRHa for subsequent frozen embryo transfer or had their cycle cancelled. Frozen embryo transfers up to one year after the stimulation cycle were included in the analysis.
Participants/materials, setting, methods: Women aged 18-40 years were randomised for ovarian stimulation to either an individualised fixed-dose of follitropin delta based on AMH and body weight (n=78) or to 150 IU follitropin alfa (n=75) which daily dose could be adjusted after 5 days of stimulation. Only single blastocyst transfers were performed with the exception of one double blastocyst transfer in the follitropin alfa group. Continuous endpoints were evaluated using t-tests. Binary endpoints were evaluated using chi-square tests.
Main results and the role of chance: Women with polycystic ovaries had the same characteristics in both treatment groups with an overall age of 31.8 years, body weight of 62.8 kg, and AMH of 49.2 pmol/l. At the end of stimulation the number of follicles (≥12 mm) was 12.1 ±7.0 and 18.3 ±7.0 with increased progesterone (>3.18 nmol/l) in 27% and 67% in women treated with individualised follitropin delta and conventional follitropin alfa, respectively. The overall mean (SD) number of oocytes was 9.3 ±6.7 and 17.9 ±8.7 respectively and there were slightly more women with blastocyst transfer (74.4% vs. 68.0%) and slightly less women with at least one cryopreserved blastocyst (61.5% vs 76.0%) in the follitropin delta group. The ongoing pregnancy rate per started stimulation cycle following fresh blastocyst transfer was 28.2% and 24.0%, respectively. The incidence of all cases of OHSS was three times higher in the follotropin

alfa group i.e. 5.1% vs 16.0% (P=0.025) as well as the combined early moderate/severe OHSS and/or preventive interventions for early OHSS i.e. 7.7% with individualised follitropin delta and 26.7% with conventional follitropin alfa (P=0.001).
Limitations, reasons for caution: This retrospective analysis includes ovulatory women with polycystic ovaries defined as serum AMH > 35 pmol/l. Anovulatory women may have different efficacy and safety profiles and should be studied separately.
Wider implications of the findings: The results demonstrate that individualised follitropin delta treatment provides an improved balance between efficacy and safety in potential high responders, as it normalizes the ovarian response and decreases the incidence of OHSS and preventive interventions of OHSS, with less embryo transfer cancellations.
Trial registration number: NCT01956110

P-593 Effect of AMH, age and history of previous pregnancy on the number of total and mature oocytes obtained in a donation programme

V. Pataia[1], M. Wolska[1], J. Wang[2], N. Macklon[2], K. Ahuja[2]

[1]London Egg Bank, London Egg Bank, London, United Kingdom ;
[2]London Women's Clinic, London Women's Clinic, London, United Kingdom

Study question: In an oocyte donation programme, does Anti-Mullerian hormone (AMH), age and/or history of previous pregnancy predict the number of total and mature oocytes obtained?
Summary answer: AMH correlated to the number of total and mature oocytes obtained from oocyte donors, but age and history of previous pregnancy did not.
What is known already: A relationship between age, AMH and ovarian stimulation response is well established in the subfertile population. However, the value of these markers in healthy oocyte donors remains to be clarified. Additionally, the effect of previous donor pregnancy on oocyte donation outcomes remains largely unexplored.
Study design, size, duration: Retrospective cohort analysis of 223 oocyte donors. Donors were aged 18-35, registered in accordance with the Human Fertilisation and Embryology Authority (HFEA) guidance, and underwent their first donation cycle between January 2018 and May 2019. Donors were divided into 6 age groups: 18-20 (n=28), 21-23 (n=60), 24-26 (n=39), 27-29 (n=45), 30-32 (n=27), 33-35 (n=24). Donors were also divided according to their reproductive history as previously pregnant (n=65) or never pregnant (n=158).
Participants/materials, setting, methods: Kruskal-Wallis test was applied to compare the relationship between study parameters and outcomes between age groups. The respective associations within each age group were investigated using Spearman's rank correlation. Mann-Whitney U test was performed to assess the effect of previous pregnancy on study parameters and outcomes.
Main results and the role of chance: No significant differences were obtained in mean AMH levels, total oocytes or total mature oocytes between donor age groups (p>0.05). Moreover, a history of previous pregnancy had no significant impact on study parameters (p>0.05). AMH was however positively correlated with the number of mature oocytes obtained (p≤0.05), and the strength of association increased with age. Similar findings applied to the total number of oocytes obtained, particularly in donors ≥27 years old.
Limitations, reasons for caution: This is a small study and a higher number of donors included would allow clinical outcomes to be assessed. All donors accepted on the donation programme had a minimum AMH level of 12pmol/L and/or antral follicle count of 12. Thus, our results may not be replicated in the general population.
Wider implications of the findings: In a cohort of healthy oocyte donors under 35 years-old meeting standard ovarian reserve criteria for acceptance, a similar number of total/mature oocytes is likely to be obtained irrespective of age or history of previous pregnancy.
Trial registration number: not applicable

P-594 Prediction of oocyte maturation rate in antagonist flexible IVF protocol using a novel machine learning algorithm

O. Houri[1], A. Wertheimer[1], Y. Gil[2], S. Danieli-Gruber[1], G. Oron[1], O. Sapir[1], S. Yoel[1], A. Ben Haroush[1]

[1]Rabin Medical Center-Beilinson Hospital, IVF and Infertility Unit, Petach Tikva, Israel ;
[2]Tel Aviv University, Electrical engineering, tel aviv, Israel

Study question: Is there a machine learning algorithm that can predict oocyte maturation rate in GnRH antagonist cycles using baseline and treatment characteristics
Summary answer: Machine learning algorithms can predict oocyte maturation rate using simple parameters. We demonstrated an accuracy rate of 75% in predicting high oocyte maturation rate
What is known already: GnRH antagonist cycles may be associated with heterogeneous follicular development that may result in lower oocyte maturation rate. Oocyte maturation is affected by different baseline and treatment parameters, and has a key effect on treatment outcome. As far as we know, a prediction model for oocyte maturation rate made by machine learning and neural network algorithms was not described yet.
Study design, size, duration: A retrospective cohort study of 462 women aged ≤ 38 who underwent their first IVF treatment using a GnRH antagonist protocol in one tertiary hospital. All were treated with a flexible antagonist protocol. Median maturation rate was approximately 80%,. Treatment parameters of cycles with high oocyte maturation rate (≥80%, n=236) were compared to cycles with low oocyte maturation rate (<80%, n=226). Patients that their oocytes were fertilized by insemination only without ICSI were excluded.
Participants/materials, setting, methods: We used XGBoost algorithm that fits the training data using decision trees, and can also rate the factors according to their influence on the prediction. We assigned a weight to each class (positive/negative), to overcome potential biases due to the unbalanced nature of data. For the machine learning training phase, 80% of the cohort was randomly selected. We have then used the rest of the samples as a test-set, to evaluate our model's accuracy.
Main results and the role of chance: The following data was retrieved from medical records: patient's age, gravity, parity, BMI, Infertility cause and duration, gonadotropins doses number of oocyte retrieved, treatment parameters on the day of GnRH antagonist initiation including estradiol and progesterone levels, maximal follicle diameter and number of follicles, GnRH antagonist treatment duration, and treatment parameters on trigger day including number of follicles, estradiol and progesterone levels.
The test-set samples' parameters were fed into the trained model and the predicted outcome was compared to the actual outcome. On the randomly picked samples used to evaluate our model, we demonstrated an accuracy rate of 75% in predicting oocyte maturation rate in GnRH antagonist cycles. The most predictive parameters arranged by descending importance order were: estradiol level on trigger day, estradiol level on antagonist initiation day, average gonadotropins units per day, progesterone level on trigger day, patients' weight, number of oocytes retrieved, patients' height, number of follicles on GnRH antagonist initiation day, patients' age and infertility duration.
Limitations, reasons for caution: Retrospective design and lack of information regarding follicle synchronization.
Wider implications of the findings: A state-of-the-art machine learning algorithm presented promising ability to predict oocyte maturation rate using simple parameters**.** We now plan to expand the cohort and evaluate the model in larger numbers
Trial registration number: not applicable

P-595 Progestin Primed Ovarian Stimulation is an effective oral alternative for Antagonist Protocol in patients undergoing assisted reproductive techniques: a retrospective study.

J.P. Caetano[1], L. Calazans[1], L. Amorim[1], E. Xavier[1], A.L. Campos[2], B. Coimbra[3], R. Marinho[1]

[1]Pro-Criar Medicina Reprodutiva, Medical, Belo Horizonte, Brazil ;
[2]Pro-Criar Medicina Reprodutiva, Embriology, Belo Horizonte, Brazil ;
[3]Hospital das Clíncias - Universidade Federal de Minas Gerais, Medical Residency, Belo Horizonte, Brazil

Study question: Are Clinical Pregnancy Rates in Progestin Primed Ovarian Stimulation noninferior to Antagonist Protocol in patients undergoing assisted reproductive techniques (ART)?
Summary answer: Our results showed noninferiority of Clinical Pregnancy Rates in Progestin Primed Ovarian Stimulation compared to Antagonist Protocol with blastocyst transfer and freeze-all strategy.

What is known already: The blockade exerted by progestin in ovulation induction involves blocking the hypothalamic-pituitary-ovary axis, changing the frequency and amplitude of GnRH pulses (La Marca et al., 2019). Early LH peak suppression occurs despite high oestradiol concentration (Kuang et al., 2015). In the current stage of ART development, in which embryo freezing is an increasingly common practice and provides good results, new options for controlling the LH peak can be considered (Kuang et al., 2015). The use of progestins has aroused interest in this regard, and the possible negative effect on the endometrium is no longer a concern (Yu et al., 2018).

Study design, size, duration: A retrospective study included all ovarian punctures performed at Pró-Criar Medicina Reprodutiva, Belo Horizonte, Minas Gerais, Brazil, between May 2018 and May 2019 using a GnRH antagonist analogue or oral progestins to block the LH peak in IVF/intra-cytoplasmic sperm injection (ICSI) cycles for infertility treatment. A total of 266 IVF/ICSI cycles performed were analysed and 222 cycles (83.5%) were included in the study, 112 in the Progestin group and 110 in the Antagonist Group.

Participants/materials, setting, methods: Exclusion criteria were age older than 42 years, fresh embryo transfer, transfer at the cleavage stage (D2/D3), preimplantation genetic screening, cryopreservation of oocytes, shared donation and cycles without embryo transfer at the time of analysis. The primary outcome evaluated was the clinical pregnancy rate at the first embryo transfer. The secondary outcomes were the mean MII oocytes retrieved, fertilization rate, blastocyst formation rate, mean duration of stimulation and mean dose of gonadotropins.

Main results and the role of chance: The primary outcome of our study, Clinical Pregnancy Rate at the first embryo (Blastocyst) transfer, was 58.4% in the progestin group and 54.9% in the antagonist group (P = 0,735), a finding consistent with most studies published to date using different progestins. The mean number of retrieved oocytes was 11 in the antagonist group and 9 oocytes in the progestin group (P = 0.009). The fertilization rate was 80% for both groups (P=0.935). The rate of blastocyst formation per cycle was 50% in the antagonist group and 55.6% in the progestin group (P = 0.106). Stimulation lasted a mean of 10 days in the two analysed groups and did not vary with patient age in either group. The gonadotropin dose used was higher in the antagonist group (2025 IU) than in the progestin group (1950 IU) (P = 0.057). In addition, the blockade was effective: there was only one case of spontaneous ovulation, which corresponded to less than 1% of the cycles, an incidence compatible with the 0.34 to 8% risk described in the literature for failure to control the LH peak in antagonist protocol cycles.

Limitations, reasons for caution: This was not a randomized controlled trial, and the choice of protocol depended on the attending physician, yet statistical analysis showed that the studied populations were similar, with similar mean age, BMI, duration of infertility and distribution of causes of infertility between the groups.

Wider implications of the findings: The use of progestins to block the LH peak has the advantages of ease of oral administration and lower cost compared to GnRH antagonists. It allows flexible ovulation monitoring, therefore more comfortable for the patient. The freeze-all strategy allows the embryos to be transferred into a more physiological uterine environment.

Trial registration number: not applicable

P-596 Salivary progesterone - a new diagnostic tool for luteal phase monitoring in Assisted Reproductive Technology (ART)

B. Alsbjerg[1], D. Sakkas[2], B. French[2], H.O. Elbaek[1], B.B. Povlsen[1], R.J. Laursen[1], M. Alper[2], P. Humaidan[1]

[1]Regional Hospital Viborg - Skive Sundhedshus, The Fertility Clinic- Skive Regional Hospital, Skive, Denmark ;
[2]Boston IVF, Boston IVF, Waltham MA, U.S.A.

Study question: Can salivary progesterone (P4) measurement be used as an alternative and more patient-friendly diagnostic tool for luteal phase monitoring during ART?

Summary answer: Luteal phase deficiency was detected by constant low salivary P4 in IVF.

What is known already: The reproductive outcome of ART depends on P4 levels during the luteal phase. Traditionally, P4 levels are measured in serum, which is cumbersome for patients due to constant venopunctures. Furthermore, when P4 level is measured in serum it is total P4; meaning, both the part tightly bound to proteins and the free bioactive portion. Free P4 is the active part accounting for only 2 % of total serum P4. Importantly, salivary P4 contains free progesterone, only.

Moreover, salivary P4 fluctuates during the natural cycle, whether this is the case in different ART cycles is unknown until now.

Study design, size, duration: An observational study including 20 patients. Ten IVF patients treated with GnRH-antagonist protocol and 10 patients undergoing Hormone Replacement Therapy Frozen Embryo Transfer (HRT-FET) were included from October 2018 to April 2019. Salivary P4 was measured over a 12-hour period from 7 a.m. to 7 p.m. on blastocyst transfer day and in HRT-FET patients on 6th day of vaginal progesterone treatment. Furthermore, serum P4 was measured the same day between 11 a.m. and 1 p.m.

Participants/materials, setting, methods: Patients were included from a Danish public fertility clinic. Saliva samples were frozen upon collection and P4 levels were measured using enzyme immunoassay kits (Salimetrics™) at a separate clinic. Immunoassay measurements were conducted precisely according to assay specifications. Reactions were conducted in hormonespecific antibody-coated microtitre plates. Samples and controls were incubated simultaneously with competitive horseradish-peroxidase-bound P4. Competitive reactions were detected using the peroxidase substrate tetramethylbenzidine (TMB) via optical density readings at 450 nm.

Main results and the role of chance: The 12-hour salivary P4 profiles in IVF patients showed substantial fluctuations on the blastocyst transfer day; however, no time related pattern was discovered. The percentage variation in IVF cycles ranged from 49 to 351%, with levels ranging from 202 pg/ml to 23953 pg/ml. One IVF patient had a mean salivary P4 level of 355 ±97 pg/ml which correlated to a low mean serum P4 level of 8.5 ng/ml. The corpus luteum (CL) deficiency was found despite the fact that the patient had 19 follicles and 12 oocytes retrieved; this patient did not conceive.

In HRT-FET cycles, similar fluctuations of salivary P4 levels were seen despite the absence of a CL and the fact that P4 derived from vaginal P4 application, only. The percentage variation ranged from 99 to 395 %, and salivary P4 levels from 8 to 21044 pg/ml. In contrast, the percentage variation in serum P4 ranged from 11 to 47 %, only.

In IVF as well as in HRT-FET cycles the ratio of salivary P4 to serum P4 varied significantly between patients. This individual variation was also previously described during natural cycle.

Limitations, reasons for caution: This study included a limited number of patients, and seven out of 160 salivary P4 levels were very high. Until now it is unclear whether these high measurements are caused by contamination of the saliva samples, due to non-compliance with collection instructions, or a true high salivary P4.

Wider implications of the findings: The analysis of salivary P4 may be a new patient friendly alternative to serum sampling for luteal phase monitoring in ART. However, future studies should explore optimal cut-off levels of salivary P4 levels during luteal phase in ART. In the long term a home test could be developed.

Trial registration number: ClinicalTrial.gov no.: NCT03725904

P-597 Cumulative live birth rate with recombinant follicle stimulating hormone biosimilar: a multicenter study with over than 7,000 cycles.

E. Arbo[1], B. Paul[2], C. Avril[3], S. Hamamah[4], J.L. Pouly[5], M. Grynberg[6], B. Salle[7]

[1]Medical Director- Gedeon Richter France, Medical Affairs Department, Paris, France ;
[2]Centre Hospitalier Universitaire de Nantes- CRTI- UMR 1064- INSERM- Université de Nantes- ITUN- CHU de Nantes, Department of Biology and Reproductive Medecine-, Nantes, France ;
[3]Clinique Mathilde, AMP Mathilde, Rouen, France ;
[4]Arnaud de Villeneuve Hospital-CHU Montpellier - INSERM U 1203- Hôpital Saint Eloi- 34295, ART-PGD Department, Montpellier, France ;
[5]CHU de Clermont-Ferrand- 63003, Gynécologie Obstétrique et Reproduction Humaine, Clermont-Ferrand, France ;
[6]Hôpital Antoine Béclère- Inserm U1185 Université Paris-Sud- Université Paris Saclay- 94276 Le Kremlin Bicêtre- France. Inserm U1133 Université Paris Diderot- 75013 Paris- France., Department of Reproductive Medicine and Fertility Preservation-, Paris, ;
[7]Hôpital Femme Mère Enfant Bron- 69677, Human Reproduction Department, Lyon, France

Study question: In routine clinical practice, is the cumulative live birth rate (cLBR) followed by IVF/ICSI using Bemfola®, a biosimilar of follitropin alfa, comparable to the originator?
Summary answer: This real-world data confirms the clinical effectiveness of Bemfola® for controlled ovarian stimulation (COS) for IVF/ICSI.
What is known already: Although randomised controlled phase 3 clinical trials on selected populations demonstrate the efficacy of new drugs to allow approval for routine use, once drugs have been introduced into clinical practice, real world data (RWD) may assess their actual effectiveness. As in the case of Bemfola®, a follitropin alfa biosimilar approved on the basis or its proven efficacy related to the number of oocytes retrieved, RWD may assess additional parameters of clinical interest such as live birth rates following IVF/ICSI. Indeed, RWD reflect the actual effectiveness in an unselected infertile population across multiple indications, protocols and clinical strategies.
Study design, size, duration: National, multicenter, retrospective, cohort study conducted in 17 public and private ART centers. Data from all cycles of women who underwent COS with a follitropin alfa, between January 2016 and February 2017 including follow-up period up to December 2017 were collected. The main assessment criterion was cumulative live birth rate (cLBR) including fresh and frozen-thawed embryo transfers per initiated cycle.
Participants/materials, setting, methods: Data from 9,162 COS cycles were extracted. The present descriptive analysis includes 7,448 cycles for which COS was performed with a follitropin alfa without any association with other gonadotropin. From these, 2,478 cycles were performed with Bemfola® and 4,970 with another follitropin alfa (90% corresponding to the originator Gonal-f®). cLBR in both groups are displayed with their 95% confidence interval (95%CI).
Main results and the role of chance: Mean female age were 34.1±4.7 and 33.9±4.7 years and mean BMI were 23.9±4.7 and 23.8±4.6 kg/m² in the Bemfola® and other follitropin alfa groups, respectively. The medians of AMH serum levels were 2.3 ng/mL (interquartile interval:1.3-3.8) and 2.6 ng/mL (interquartile interval: 1.4-4.7) and the proportion of women presenting an AMH serum level > 5.2 ng/mL were 14.8% and 21.5%, in the Bemfola® and other follitropin alfa groups. The most prescribed starting dose of follitropin alfa were between 150-225 IU in both groups. Oocyte retrieval resulted in 7.1±5.7 and 7.9±5.5 mature oocytes in the Bemfola® and in the other follitropin alfa groups, respectively. cLBR per cycle were 20.0% (95% CI: 18.4%-21.5%) in the Bemfola® group and 20.8% (95% CI: 19.7%-21.9%) in the other follitropin alfa group. cLBR in the COS cycles that were followed by at least one embryo transfer during the follow-up period were 21.2% (95% CI:19.5-22.8) in the Bemfola® group and 21.6% (95% CI:20.5-22.8) in the other follitropin alfa group.
Limitations, reasons for caution: Although systematic biases cannot be excluded, detailed analyses revealed that the apparent biases seem to favor the originator follitropin alpha, suggesting that Bemfola® had been preferentially prescribed to poorer prognosis patients. The comparator follitropin alpha group included another follitropin alpha biosimilar, but too few cases to impact results (10%).
Wider implications of the findings: This is the largest multicentric cohort describing cLBR with the use of a follitropin alfa biosimilar in a non-selected population. This real-world evidence demonstrates that the use of Bemfola® is an effective option for COS, with clinical results comparable with other follitropin alfa.
Trial registration number: NA

P-598 The Effects of Lipid Metabolism and Obesity on the Number of Retrieved Oocytes in Polycystic Ovary Syndrome Undergoing Natural Cycles

T. Liu[1], J. Yan[1]

[1]Beijing University Third Hospital, Center for Reproductive Medicine, Beijing, China

Study question: To assess the effects of lipid metabolism and body mass index (BMI) on the number of retrieved oocytes (no.RO) in polycystic ovary syndrome (PCOS) undergoing natural cycles.
Summary answer: The increase of serum low density lipoprotein cholesterol (LDL-C) can improve anti-Mullerian hormone (AMH) level, antral follicle counts (AFC) and no.RO when BMI is normal.
What is known already: The no.RO can be affected by various factors in controlled ovarian hyperstimulation (COH) cycles, in which BMI and lipid metabolism are very important to predict ovarian response.
Study design, size, duration: This is a retrospective cohort study. None interventions were applied to the patients. The main outcome measures are as followed: Lipid metabolic index, including total cholesterol (TCHO), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and LDL-C. Basal sex hormone, including follicle stimulation hormone (FSH), luteinizing hormone (LH), progesterone(P), estradiol (E2) and AMH. IVF index, including AFC, no.RO, maturation rate (MR), fertilization rate (FR) and embryo formation rate (EFR).
Participants/materials, setting, methods: The participants were PCOS patients with clomiphene resistance undergoing natural cycles from January 1, 2015 to November 30, 2019. All the data were from clinical records.
Main results and the role of chance: 219 patients were eventually included in this study. Scatter plots and fitting curves showed that TCHO and LDL-C had a tendency to increase first and then decrease with no.RO, while HDL-C had a tendency to decrease first and then increase with no.RO. The correlation between BMI, TG and no. RO was not statistically significant. Cutoff values of TCHO (5.89mmol/L), HDL-C (1.71mmol/L) and LDL-C (4.09mmol/L) were identified through fitting curves. Among four BMI groups (low: <18.5Kg/m2, normal: 18.5~24Kg/m2, overweight: 24~28Kg/m2 and obesity: ≥28Kg/m2), only AMH and four lipid metabolic index showed statistically significant differences, while all IVF index and other basal sex hormone did not. Next, cutoff values were used separately to divide each BMI group into two subgroups. The results showed that significant differences were found in AMH, AFC and no.RO between LDL-C subgroups (BMI: normal), P and AFC between TCHO subgroups (BMI: normal), E2 between LDL-C subgroups (BMI: overweight) and P between LDL-C subgroups (BMI: obesity). No statistically significant differences in MR, FR and EFR were observed in all subgroups comparisons.
Limitations, reasons for caution: The sample size of subgroups higher than TCHO, HDL-C and LDL-C cutoff values is still limited, and this part of the sample size should be further expanded.
Wider implications of the findings: LDL-C may be a better predictor of no.RO than BMI in natural cycles.
Trial registration number: ChiCTR-ONC-17011861

P-599 Replacing antagonist by clomiphene citrate to prevent premature ovulation in IVF-ET: is it possible?

S. Ekmekjian[2], H. Maroun[1], C. Boyadjian[1], T. Tadros[1], I. Aboujaoude[1]

[1]Aboujaoude Hospital-, Center for Reproductive Medicine-, jaleldib, Lebanon ; [2]Lebanese University faculty of sciences II, Reproduction biology lab, Fanar, Lebanon

Study question: Do 150 mg of clomiphene citrate (CC) capable of replacing the classical antagonists in controlled ovarian stimulation (COS) to prevent premature ovulation (PO)?
Summary answer: Our data indicated that CC is as efficient as antagonist in IVF-ET.
What is known already: Since the introduction of antagonists in the 90th, the antagonist protocol is becoming the most used protocol in COS for IVF-ET patients. However, due to their relatively high cost, infertility specialists in developing countries try to find a more affordable molecule to avoid premature the ovulation during COS. New larges studies showed that CC could be used to prevent PO in mild stimulation using 50 mg daily. No studies to our knowledge used CC in standard IVF-ET protocols. Therefore, we decided to do a pilot study in order to replace antagonists with CC 150 mg per day until triggering.
Study design, size, duration: We studied 161 patients who entered our IVF-ET program. All patients started COS on day 3 of the menstrual cycle using HMG or recombinant FSH.
Participants/materials, setting, methods: Patients were sorted as follows: group 1 Ganirelix (n=73) underwent the classic antagonist protocol where Ganirelix was introduced at day 6 of the COS; group 2: CC (n=88) where we replaced the antagonist by CC 150 mg. Single or double triggering for final oocyte maturation was performed using HCG 5000 IU and/or Decapeptyl 0.3 mg when at least 3 follicles reached 17mm in diameter. Oocyte retrieval was conducted 35 to 37 hours after triggering.
Main results and the role of chance: As shown in the Table1, whereas patients and COS characteristics were similar in both groups, we observed no statistical differences in terms of number of oocytes retrieved, empty follicles cases and pregnancy rates.

		ANT CC(n=73)	ANT Org(n=88)	P
Ages (years)		28.1 ± 4.71	30.3 ± 4.39 (19-35)	0.0032*
Duration of infertility (years)		7.3 ± 4.034 (0.5-19)	6.8 ± 4.348	NS
IVF-ET Indications	• **Male (%)**	68.5	65.5	NS
	• **IUI failure (%)**	12	13.5	NS
	• **Tubal (%)**	11.5	11	NS
	• **Endometriosis (%)**	8	9	NS
Duration of COS (days)		8.4 ± 1.44	8.6 ± 1.89	NS
E2 day of trigger (pg/ml)		2825.5 ± 2065	1870.4 ± 1414.60	0.01
Progesterone day of trigger >1.5 ng/ml (%)		21.1	16.7	NS
Total dose of Gonadotropins (IU)		2108.6 ± 988.15	1954.5 ± 737.72	NS
No. of total oocytes		9.8	11.3	NS
MII oocytes (%)		69.3	77	NS
Empty follicles (%)		4.1	2.3	NS
No. of embryos transferred		2.7	2.5	NS
Clinical pregnancy rate / transfer (%)		38.8	24.7	NS
Ongoing pregnancy / transfer (%)		31.3	23.5	NS
Miscarriage rate (%)		19.2	5	NS

38.831.319.2

Limitations, reasons for caution: The present study could not address the exact mechanism of action of the CC on the hypothalamic-pituitary axis to prevent PO neither discriminating possible outcome differences using lower dose of CC due to limited sample size. Moreover, potential side effects of CC where not taken into consideration in this study.

Wider implications of the findings: Our data indicate that clomiphene seems to be as efficient as the antagonists, cheaper, and easier to use in order to prevent premature ovulation in IVF-ET. Further randomized controlled studies, including larger populations, are needed to confirm and expand present findings**.**

Trial registration number: not applicable

P-600 Transdermal testosterone vs. oral dehydroepiandrosterone (DHEA) pre-treatment in improving IVF outcomes in diminished ovarian reserve patients (POSEIDON group 3 and 4): a randomised controlled trial

N. Sharma[1], K.D. Nayar[1], S. Gupta[2], M. Singh[1], R. Bhattacharya[1], G. Kant[1], R. Gahlot[1], K.D. Nayar[1]

[1]Akanksha IVF Centre- Mata Chanan Devi Hospital, Reproductive Medicine, New Delhi, India ;

[2]Akanksha IVF Centre- Mata Chanan Devi Hospital, Reproductive Medicine, New Delhi, India

Study question: To compare the efficacy of transdermal testosterone with oral dehydroepiandrosterone in improving IVF outcomes using GnRH antagonist protocol in POSEIDON group 3 and 4 patients.

Summary answer: Patients receiving pre-treatment with testosterone gel had higher mean number of oocytes retrieved and grade A embryos as compared to the patients receiving oral DHEA

What is known already: Diminished ovarian reserve (DOR) is associated with suboptimal ovarian response, higher cycle cancellation rate and lower clinical pregnancy rate following IVF cycles. Various treatment regimens have been devised for management of such patients and use of adjuvants in the form of oral or transdermal androgen is one of them. Androgens improves follicular response to gonadotropin stimulation as well as increase FSH receptor expression in granulosa cells, in turn leading to better oocyte yield and pregnancy rate. Aim was to compare the effect of transdermal testosterone gel with oral DHEA on the ART outcome in DOR patients(POSEIDON Group 3 and 4)

Study design, size, duration: A prospective, randomised controlled trial was carried out from 1st January 2019 to 31st October 2019 at a tertiary infertility centre in India. 50 patients fulfilling the criteria of Group 3 and Group 4 of POSEIDON classification were included in the study. Patients with endocrine disorders (thyroid, prolactin), endometrioma, history of surgery on the ovaries, sensitivity to testosterone gel, male factor infertility and deranged liver and renal function tests were excluded.

Participants/materials, setting, methods: Enrolled patients were randomised into two groups of 25 patients each, one group was pretreated (TTG group) with transdermal testosterone gel ,12.5 mg/day from day 6th of previous cycle to day 2nd of stimulation cycle while patients in other group took DHEA tablet,75 mg/day for a total duration of three months (DHEA group) before stimulation with GnRH antagonist fixed protocol followed by fresh Day 3 transfer

Main results and the role of chance: The baseline characteristics of the two groups were comparable. The primary outcome measures were the number of oocytes retrieved and number of grade A embryos formed (according to Istanbul consensus). The secondary outcome measures were implantation rate, clinical pregnancy rate, miscarriage rate and ongoing pregnancy rate. The mean number of oocytes retrieved in TTG group was 5±1.4 which was significantly higher than DHEA group-3.3±1.7, ($p<0.001$). The mean number of Grade A embryos were also significantly higher (4.28±0.88 vs 2.85±0.63, $p<0.001$) in TTG group. The TTG group had higher implantation rate (28% vs 20%, $p= 0.49$), clinical pregnancy rate (32% vs 20%, $p = 0.41$), ongoing pregnancy rate (32% vs 16 %, $p= 0.38$) and lower miscarriage rate (0% vs 20%, $p=0.38$), however, these differences were not statistically significant.

Limitations, reasons for caution: The study was done at a single centre with small sample size, replication with more subjects and in different centers is needed. Also, cost effectiveness of either drug was not assessed.

Wider implications of the findings: Pre-treatment with testosterone gel in DOR patients improves ovarian response to stimulation and results in higher number of oocytes retrieved and good quality embryos resulting in improved clinical pregnancy rates. Transdermal testosterone is advantageous because of better bioavailability, easy application, patient friendly and less adverse effects.

Trial registration number: MCDH/2019/28

P-601 The role of midkine, a heparin-binding growth factor, in human follicle development and oocyte maturation

S.E. Pors[1], S.G. Kristensen[1], D. Nikiforov[1], L. La Cour Poulsen[2], A. Sanchez[3], I. Pla Parda[3], C. Yding Andersen[1]

[1]Rigshospitalet, Laboratory of Reproductive Biology, Copenhagen, Denmark ;
[2]Zealand University Hospital, Zealand Fertility Clinic-, Køge, Denmark ;
[3]Lund University, Department of Translational Medicine, Lund, Sweden

Study question: Does midkine, a heparin-binding growth factor found in follicle fluid, affect human follicle development and oocyte maturation?
Summary answer: Midkine affect human follicle development by augmenting oocyte maturation *in vitro* and its concentration increases in intrafollicular fluid during the mid-cycle surge of gonadotropins.
What is known already: In vitro maturation (IVM) of immature oocytes without exogenous FSH stimulation has attracted much interest in recent years and immature oocytes from small antral follicles (SAF) is a new way of augmenting the fertility potential of women undergoing fertility treatment by having ovarian tissue cryopreserved. However, regulation of human oocyte maturation is highly complex and dynamic and far from fully elucidated. The growth factor midkine, acting via the NOTCH signalling pathway, has recently been identified in human follicular fluid (FF) and may represent an important factor for follicle and oocyte development.
Study design, size, duration: Follicle fluid (n=50) from SAF, immature oocytes (n=891) and tissue were obtained from ovaries from women undergoing ovarian tissue cryopreservation for fertility preservation. Small antral follicles with different diameters were isolated from surplus medulla tissue (n=183). In addition, FF from preovulatory follicles were aspirated from women undergoing fertility treatment at different time points after final follicle maturation was induced (n=25). All women had given written consent for use of the material for research purposes.
Participants/materials, setting, methods: Concentration of midkine in FF was analysed by proteomics and ELISA. Microarray data on gene expression from SAF were obtained and analysed. Immunohistochemistry using antibodies against midkine and Notch2 were performed. Immature oocytes, collected after ovarian tissue cryopreservation, were randomly allocated for IVM with or without midkine in the media (1 ug/ml). From the same patient, two FF containing immature oocytes from SAF were obtained. FF levels of midkine were associated to the IVM outcome.
Main results and the role of chance: FF samples from preovulatory follicles were collected at five time points after ovulation trigger: 0, 12, 17, 32 and 36 h, respectively. Expression of midkine protein was significantly higher at 0,12 and 17 h compared to 32 and 36 after the ovulation trigger. When measuring the midkine content with ELISA the concentration of midkine was high at all timepoints measured after administration of the ovulation trigger and only decreased significantly at 32 h. In SAF midkine was also present in concentration ranging from 190-2300 ng/ml. The highest levels of gene expression of midkine was present in SAF (<9 mm) compared to larger follicles (>9 mm). Preliminary results of the immunohistochemical investigations indicate that midkine and its potential receptor, NOTCH2, both are present in granulosa and cumulus. In addition, midkine significantly increased the maturation rate of immature oocytes (without midkine: 27%; with midkine: 34%, p=0.04). The concentration of midkine was compared using ELISA between FF from SAF from the same patient. Follicles that contained an oocyte that matured contained higher concentration of midkine as compared to the follicle in which the oocyte failed to mature (n=16 follicles from 8 patients).
Limitations, reasons for caution: The dynamics in the preovulatory follicles were investigated in FF from follicles obtained in stimulated cycles and the use of ovulation trigger may have affected results.
Wider implications of the findings: These results indicate that midkine is a new growth factor, important for human follicular development both during ovulation and in folliculogenesis. Further investigations on the processes taking place in the ovary, will increase the possibility for optimizing and developing future treatments especially for women with a low ovarian reserve.
Trial registration number: Not applicable

P-602 Comparison of letrozole versus clomiphene citrate (CC) for ovulation induction in infertile women with polycystic ovary syndrome (PCOS) in Indian population: A prospective clinical trial

E. Gupta[1], K.D. Nayar[1], S. Gupta[1], M. Singh[1], R. Bhattacharya[1], R. Gahlot[1], G. Kant[1], K.D. Nayar[1]

[1]Akanksha IVF Centre- Mata Chanan Devi Hospital, Reproductive Medicine, New Delhi, India

Study question: To compare the efficacy of letrozole and clomiphene citrate for ovulation induction in infertile women with PCOS for IUI cycles in Indian population
Summary answer: Letrozole favors monofollicular development and has a better endometrial response when compared to CC though no difference in the clinical pregnancy and multiple pregnancy rate
What is known already: Clomiphene citrate is used for ovulation induction in PCOS. Clomiphene resistance occurs in 15-20% of the patients. PCOS women in India have a higher prevalence of insulin resistance(~75%) and are more likely to have CC resistance. CC also has a negative effect on the cervical mucus and endometrium. Treatment with CC is thus associated with a discrepancy between ovulation and conception rates. Letrozole, an aromatase inhibitor used for ovulation induction has no adverse effects on the endometrium and the cervical mucus. It can thus act as an effective alternative to CC in Indian women with PCOS
Study design, size, duration: A prospective clinical trial on 120 infertile patients with PCOS diagnosed according to Rotterdam criteria was carried out at a tertiary care infertility centre in India from January 2019 – October 2019.
Participants/materials, setting, methods: One hundred and twenty women with PCOS were divided into two groups-61patients with Letrozole, 2.5 mg/day and 59 patients with clomiphene citrate 100 mg/day from day 3-7 of the menstrual cycle. Follicular monitoring was done and 10,000 IU of HCG was administered when the largest follicle was ≥18 mm. Intra-uterine insemination was done 36-40 hours after HCG administration. 400mg micronized progesterone was given intra-vaginally for 15 days as luteal phase support.
Main results and the role of chance: Baseline characteristics in both letrozole and CC group were comparable. Mean number of follicles ≥ 18mm on the day of hCG administration was 1.13±0.53 in letrozole group and 2.6±1.15 in the CC group (p <0.0001). Mean endometrial thickness on the day of hCG administration was 8.21±0.86 in letrozole group and 7.35±0.99 in CC group (p <0.0001). Ovulation rate in letrozole group was 77.04% and in CC group was 59.32%, which was not statistically significant (p= 0.05). Clinical pregnancy rate was 14.75%(9/61) with letrozole group and 13.56%(8/59)with CC group, however this difference was not statistically significant (p>0.05). Mutiple pregnancy rate was higher with CC group (2.5% (2/8)vs. 1.11%(1/9) , p=0.57) but not statistically significant
Limitations, reasons for caution: The study was done at a single centre with small sample size. Replication with more subjects and multiple centres is needed
Wider implications of the findings: Letrozole leads to more monofollicular development and better endometrial response compared to CC. Hyperinsulinemia, which is frequently associated with PCOS, is one of the causes for CC resistance. The prevalence of insulin resistance in PCOS is approximately 75%.Thus, letrozole has an important role as first line treatment for PCOS patients.
Trial registration number: MCDH/2019/45

P-603 Drug free In Vitro Activation for woman with very advanced maternal age

L. Valkova[1], N. Magunska[2], B. Petkova[3], T. Milachich[3], K. Kawamura[4], A. Shterev[2]

[1]SAGBAL Dr Shterev EOOD, IVF Unit, Sofia, Bulgaria ;
[2]SAGBAL Dr Shterev EOOD, Department of Obstetrics and Gynecology- IVF Unit, Sofia, Bulgaria ;
[3]SAGBAL Dr Shterev EOOD, IVF Unit, Sofia, Bulgaria ;
[4]Advanced Reproductive Medicine Research Center- International University of Health and Welfare School of Medicine, Department of Obstetrics and Gynecology, Narita, Japan

Study question: What is the effect of drug-free In Vitro Activation (IVA) for woman with very advanced maternal age (vAMA)?
Summary answer: Drug free IVA had positive effect through decreasing the levels of FSH, LH, E2. Good quality oocytes were retrieved and top quality embryos were obtained.
What is known already: There are studies demonstrating good results in patients with premature ovarian insufficiency (POI) using drug- free IVA. The mechanism involves suppression of Hippo signaling pathway leading to secondary follicle growth. Women with diminished ovarian reserve (DOR) as well as patients with AMA and vAMA have very low number of follicles and oocytes

even after applying of ovarian hyperstimulation. Their chance for pregnancy and live birth is minimal but number of patients with AMA or vAMA who wish to have children with autologous oocytes increases constantly.
Study design, size, duration: Case report. From March to November 2019, after refusing the use of donor oocytes to patients with vAMA were proposed application of drug-free IVA. Detailed information for risks and chances were provided to the women and one woman signed necessary informed consents. The procedure was performed after permission of the Ethics Research Committee of the hospital.
Participants/materials, setting, methods: To a 48 years-old woman with primary infertility drug-free IVA was performed by taking pieces from both ovaries under laparoscopic surgery. The cortices were dissected into small cubes (2x2x2 mm) that were auto-transplanted at the same surgery. The ovarian pieces were placed into peritoneal pockets of the mesosalpinx and of the remaining ovaries followed by closure using sutures. Ovarian stimulation and fertilization with donor sperm of the retrieved oocytes were performed after manipulation.
Main results and the role of chance: The hormone levels before and after drug-free IVA were compared. They were: FSH – 50.8 vs 8.3 IU/L, LH - 38.1 vs 6.1 IU/L and E2 – 79.4 vs 56.9 pg/ml, respectively. Before manipulation the hormonal ovarian stimulation was impossible because of high levels of gonadotropins. After surgery, we succeeded to make hormonal stimulation three times. In each cycle we achieved: follicle growth, high quality oocytes and embryos using donor sperm. After these three stimulations, we obtained six oocytes and five embryos. Embryo transfer (ET) was performed two times: the first one on day 2 with two embryos and second one on day 3 with one embryo. All embryos were with top quality for the corresponding day, but pregnancy was not achieved. One of possible reasons for the negative result could be obstruction of the implantation by 3 myomas in the uterus of the patient. Laparoscopy for elimination of the myomas is forthcoming. Until the appropriate moment for the ET we decided to generate embryos in different stimulation cycles with subsequent cryopreservation by vitrification. We have already vitrified on day 3 two top quality embryos from the third stimulation.
Limitations, reasons for caution: The procedure has to be finished and is important if the pregnancy and live birth will be achieved. In contrast to positive effect of drug-free IVA on improvement of oocyte quality, it cannot fix the issue of aneuploidy. Additional genetic tests before implantation or after pregnancy could be performed.
Wider implications of the findings: There are data about children born after drug-free IVA in young patients. If the results are good also for patient with AMA and vAMA this could be approved as useful method for this hard for treatment steadily growing groups of patients.
Trial registration number: NA

P-604 Does the Freeze-all strategy improves the cumulative live birth rate and the time to become pregnant in IVF cycles?

A. Delvigne[1], J. Vandromme[2], A. Larbuisson[1], S. Johnson[1]

[1]CHC Montlégia, ART center, Liège, Belgium ;
[2]CHU Saint-Pierre, Gynecology, Brussels, Belgium

Study question: Does the Freeze-all strategy improves the cumulative live birth rate in IVF cycles?
Summary answer: The CLBR is significantly lower in the FA group. Nevertheless, the CLBR in the FA group remains superior to that observed in previous studies.
What is known already: Elective freezing of all good quality embryos and transfer in subsequent cycles, named as the freeze-all strategy (FAS) is widely used for ovarian hyperstimulation syndrome (OHSS) prevention. The benefits of the FAS on live birth rates among high responders have been shown by many studies. Similarly, the benefits of frozen embryo transfer (FRET) compared to fresh embryo transfer (FET) has been demonstrated to prevent preterm birth and small for gestational age. Consequently, why should we limit the FAS to high responders rather than extend it to all?
Study design, size, duration: A retrospective and monocentric study was conducted between January 2008 and January 2018 comparing the cumulative live birth rates (CLBR) of patients having undergone FAS to those using FET and having at least one frozen embryo during the same period.
Participants/materials, setting, methods: Logistic regression (LR) was used to identify confounding variables. Analysis were made for the entire cohort and also for different subgroups according to the BELRAP (Belgian Register for Assisted Procreation) criteria and to confounding factors selected by the LR.
Main results and the role of chance: 2216 patients were studied in all. 233 patients constituted the freeze all (FA) group and 1983 the control (C) group (population 1). Patients selected according to BELRAP criteria (less than 36 years old and first IVF trial) were divided as follows: 124 patients in the FA group with 1241 in the C group (population 2). For these two groups, the CLBR was respectively 50.2% vs 58.1% P=0.021 for population 1 and 53.2% vs 63.3% P=0.023 for population 2. LR revealed the following confounding variables: age, rank of the attempt, tobacco, number of oocytes retrieved, number of embryos obtained, and the date of oocytes retrieval before 2011 and after 2011. Once these confounding variables were excluded, the FA and the C group were restricted to respectively 109 and 770 patients. The CLBR stays in favour of the control group: 70.1% vs 55.9% P=0.03. The time to become pregnant is equally in favour of the C group with a median of 5 days against 61 days for the FA group.
Limitations, reasons for caution: Its a retrospective study carried over a long Long period of time with changes in the freezing techniques and in our transfer politics during the study. We also have an heterogeneous FA group with mostly hy- responders (75%).
Wider implications of the findings: The CLBR is significantly lower in the FA group compared to the control group (excellent rate of 70.1%) witch is the contrary of what is found in recent studies.
But CLBR of 55.9% is still a very good rate for the FA population as its the best found in the littérature.
Trial registration number: not appilcable

P-605 Letrozole did not improve endometrium receptivity in non-ovulation PCOS women, one RCT study for endometrium preparation before thawed embryo transfer in non-ovulation PCOS women.

C. Wang[1], Q. Mai[1], C. Zhou[1]

[1]the first affiliated hospital of Sun Yat-sen University, repeoductive medical center, Guangzhou, China

Study question: Does letrozole can improve the endometrium receptivity in women with PCOS who underwent thawed embryo transfer (TET)?
Summary answer: Letrozole did not improve endometrium receptivity and clinical pregnancy rate in non-ovulation PCOS women.
What is known already: Polycystic Ovarian Syndrome (PCOS) is the most common endocrine disorder in infertile women. For non-ovulation PCOS women, hormone replace treatments (HRT) are always used for the endometrium preparation before thawed embryo transfer. In international PCOS guideline, Letrozole has been advised as the first line controlled ovarian stimulation medicine. Letrozole is not only be used as COH medicine but also could be used as endometrium reparation before thawed embryo transfer in non-ovulation PCOS women. However, there is no evidence show the effect of letrozole on endometrium receptivity and clinical pregnancy rate in women with PCOS who undergoing TET.
Study design, size, duration: One RCT study (ChiCTR1800014746) involved PCOS women undergoing TET at Reproductive Medicine Center of The First Affiliated Hospital of Sun Yat-sen University from 2017 to 2019. The sample size was calculated based on previous pregnancy rate of letrozole COH and HRT endometrium preparation in PCOS women underwent TET. Considering the mean pregnancy, 5% type I error and 20% type II error, 130 patients were required in each group and 150 patients were studied in each group.
Participants/materials, setting, methods: 296 non-ovulation PCOS women were involved in our RCT. 148 women were randomized selected into HRT endometrium preparation group and 148 women were radonmized selected into letrozole COH endometrium preparation group.In letrozole COH endometrium preparation group,10 cycles were canceled. In HRT endometrium preparation group, 7 cycles were canceled. SPSS ver. 20 was used for dada analysis. P value of less than 0.05 was considered statistically significant with Fisher corrections.
Main results and the role of chance: Total 283 TET cycles were evaluated, 140 for letrozole COH endometrium preparation group (group A) and 143 for HRT endometrium preparation group (group B). The mean age in group A and group B were 30.09 and 29.61 years (p=0.853). The base line of basic FSH, BMI, the number of oocytes and embryo transfer number were no significant

difference between two groups. The endometrium thickness on the day endometrium transformation was significant higher in Group A.(10.28±1.94mm VS 9.21±1.59mm; P<0.001) The E2 level on embryo transfer day were significantly lower in Group A (88.6pg/ml VS 181pg/ml; P<0.01). The progesterone level on embryo transfer day were significantly higher in Group A . (23.22±12.39 VS 12.59±6.53; P<0.001) The average number of clinical visiting times was 3.1±0.89 in Group A and 2.2±0.8 in Group B. (P<0.05) Clinical pregnancy rate were 54.7% in Group A and 60.3% in Group B. (P=0.63) The endometrium grew more slowly in Group A. In Group A, the average day for endometrium thickness reaching 7mm and above was about 4.5 days before the day of endometrium transformation. However, the endometrium thickness in Group B can reach 7mm before 7 days before the day of endometrium transformation.

Limitations, reasons for caution: It is a single center RCT study and the life birth rate of two groups are not available right now. Our research did not evaluate the mechanism according to the endometrium receptivity in Letrozole COH endometrium preparation group.

Wider implications of the findings: For non-ovulation PCOS women, Letrozole COH endometrium preparation protocol can provide comparable clinical pregnancy rate. However, HRT endometrium preparation protocol could be more convenient due to less number of clinical visiting times and stable high clinical pregnancy rate. It is still beyond studies about endometrium receptivity in Letrozole COH patients.

Trial registration number: ChiCTR1800014746

P-606 Effect of body mass index on cumulative live birth rate following in vitro fertilization

L. Zhou[1]

[1]*Shenzhen Zhongshan Urology Hospital, Fertility Center, Shenzhen-Guangdong, China*

Study question: Is it beneficial to the cumulative live birth rate of in vitro fertilization by achieving a particular body mass index prior to infertility treatment?

Summary answer: BMI does not affect the cumulative live birth rate of IVF, but it does elevate the miscarriage rate in the fresh cycles.

What is known already: It is critical to evaluate the impact of body mass index on the cumulative live birth rate following in vitro fertilization, as achieving a lower BMI prior to infertility treatment is often recommended for women with overweight and obesity. It is important to extrapolate the true benefits of weight loss on CLBR from this study.

Study design, size, duration: This is a retrospective cohort study. A total of 4166 women aged 20-40 years old with first conventional stimulation across different BMIs at our institution from 2012 to 2016 were stratifified into cohorts. BMI was defifined according to World Health Organization guidelines.

Participants/materials, setting, methods: The primary outcome was live birth as measured by cumulative live birth rate per initiated cycle. Secondary outcomes included clinical pregnancy rate, miscarriage rate and live birth rate independent in fresh and cryopreserved cycles.

Main results and the role of chance: There were 3384 first fresh cycles and 1929 subsequent frozen embryo transfer cycles. There were 3645 live births, for an overall cumulative live birth rate of 87.49% per initiated cycle. The clinical pregnancy rate and cumulative live birth rate decreased with increasing BMI, but there was no statistical significance. The miscarriage rate was lower in underweight (BMI < 18.5 kg/m2) and normal weight cohorts (18.5 <BMI < 24.9 kg/m2, over weight cohort (BMI>25 kg/m2) of patients had a relatively higher rate in fresh cycles(P=0.041), but there was no statistical significance in all linked cryopreserved cycles.

Limitations, reasons for caution: Our conclusions are limited by the retrospective nature of the study, and the single-center design also weakens the universality of our observations. It is possible that patients with a failed initial transfer and subsequent weight loss, followed by a successful transfer, were not captured within this study's time frame.

Wider implications of the findings: BMI does not affect the cumulative live birth rate of IVF, but it does elevate the miscarriage rate in the fresh cycle. Therefore, it is recommended to delay embryo transfer to achieve a lower BMI may be beneficial to assisted reproductive outcomes.

Trial registration number: no

P-607 Factors in first complete in vitro fertilisation cycle which predict success in second complete cycle

X. Zhang[1], **T. Dineen**[1], **A. Kovacs**[1], **R. Mihart**[1], **J. O'Callaghan**[1], **J. Culligan**[1], **A. Tocado**[1], **N. Daly**[1], **M. Walsh**[1], **D. Florio**[1], **D. McAuliffe**[1], **J. Waterstone**[2]

[1]*Waterstone Clinic, Laboratory, Cork, Ireland ;*
[2]*Waterstone Clinic, Clinic, Cork, Ireland*

Study question: To determine the factors in first complete in vitro fertilisation (IVF) cycle which predict live birth (LB) in second complete IVF cycle.

Summary answer: The patients that had LB, miscarriage or had frozen embryos from first complete IVF cycle had a greater likelihood of achieving LB in second cycle.

What is known already: Individualised prognostics are valuable for both patients and practitioners. A better understanding of the factors that determine the chance of success in IVF can help patients choose the correct fertility treatment options.

Studies have shown that treatment response in the first cycle is reproducible. The number of aspirated oocytes is a predictor for subsequent treatment outcome. However, the majority of studies have considered only the outcome of the fresh IVF transfer without the inclusion of frozen embryo transfer results.

Study design, size, duration: This cohort study includes all women (Median age = 36.0, 19.6% ≥ 40years) undergoing IVF treatment with homologous oocytes in a single IVF unit who had a first (n= 4180) and a second (n=1838) egg collection between 2008 and 2017. The outcome of all embryo transfers (fresh and frozen) from both egg collections was analysed.

Participants/materials, setting, methods: A complete cycle is defined as all fresh and frozen/thawed embryo transfers derived from the same oocyte aspiration. LB rates of the 1st and 2nd complete cycles were calculated. Multivariate logistic regression was used to identify prognostic factors in the first complete cycle with regard to the outcome of the second complete cycle, such as the number of retrieved oocytes, number of good quality embryos, blastocyst cryopreservation and pregnancy outcome.

Main results and the role of chance: In all women LB rate was 38.0% (n=4180) for the first complete cycle, significantly higher than for the second complete cycle 34.0% (n=1838, p=0.003).

In the second complete cycle LB rate was 30.1% (364/1202) in women that did not achieve any pregnancy in the first complete cycle in comparison with 44.2% (156/353, OR=1.5) in women with LB and 37.1% (105/283, OR=1.3) in women with miscarriage. Moreover, embryo development patterns in the first complete cycle demonstrated a significant association with LB rate in the second complete cycle. A significantly higher LB rate in the second complete cycle was found for women who had frozen blastocyst(s) in the previous cycle (OR=1.4, P=0.019) compared to those who had not.

The LB rate of the 2nd complete cycle significantly increased with the number of oocytes retrieved in the first complete cycle. However, this association was only significant in the poor ovarian response group (number of oocytes less than 4) after adjusting for the factors of womens' ages and of having frozen blastocyst(s).

Limitations, reasons for caution: This is a cohort analysis based on retrospective data collection. A significant number of patients did not undergo a second cycle, which may potentially bias the results.

Wider implications of the findings: These results allow us to provide patients with more accurate advice when they have a consultation after their first complete cycle.

Trial registration number: not applicable

P-608 Optimization of oocyte retrieval rate in women undergoing ovarian stimulation for in-vitro fertilization using hCG or GnRH agonist for triggering final oocyte maturation

J. Bosdou[1], **E. Deligeoroglou**[1], **O. Pavlidi**[1], **A. Makedos**[1], **A. Mitsoli**[1], **G. Lainas**[1], **T. Lainas**[2], **I. Sfontouris**[2], **M. Malandri**[1], **T. Tarlatzi**[1], **D. Sabbaidou**[1], **A. Chatzimeletiou**[1], **L. Zepiridis**[1], **G. Grimbizis**[1], **E. Kolibianakis**[1]

[1]*Aristotle University of Thessaloniki- Medical School-, 1st Dept. of Ob/Gyn- Unit for Human Reproduction-, Thessaloniki, Greece ;*
[2]*Eugonia, Assisted Reproduction Unit, Athens, Greece*

Study question: At which follicular diameter is the ratio of oocytes retrieved to the number of follicles > certain diameter on the day of triggering final oocyte maturation (oocyte retrieval rate) optimised?
Summary answer: The smallest mean follicular diameter, leading to a lower 95% confidence-interval(CI) of oocyte retrieval rate ≥100%, is 15mm for hCG and 16mm for GnRH-agonist triggering.
What is known already: Oocyte retrieval rate is calculated by dividing the number of oocytes retrieved to the number of follicles equal or greater than a specific mean follicular diameter (i.e. ≥11mm, ≥12mm, etc.), assessed in two perpendicular planes on the day of triggering. Ideally, the smallest mean follicular diameter leading to a lower 95%CI of oocyte retrieval rate ≥100% should be used for this purpose. In this case, the number of oocytes retrieved would be expected to be ≥ to the number of follicles above this follicular diameter. Although this is an essential information for patient consultation, no such data is currently available.
Study design, size, duration: A retrospective study was performed between 01/2018 and 12/2019, including 413 in-vitro fertilization (IVF) cycles (306 women). Ovarian stimulation was performed with a fixed dose of recombinant gonadotrophin and gonadotrophin releasing hormone (GnRH) antagonist, while triggering was carried out with human-chorionic-gonadotrophin(hCG) or GnRH-agonist, when ≥14 follicles ≥11mm in diameter were present. Thirty-six hours following final oocyte maturation, oocyte pick-up(OPU) was carried out by puncturing all follicles that could be aspirated. Follicular flushing was not performed.
Participants/materials, setting, methods: Follicular development was assessed by recording the mean diameter of each follicle ≥10mm by 2-dimensional ultrasound. On the day of triggering, the number of follicles ≥ to a specific diameter was calculated for all diameters recorded, leading to multiple oocyte retrieval rates for each cycle. The primary outcome measure was the identification of the smallest follicular diameter on the day of triggering, with which the lower 95%CI of the calculated oocyte retrieval rate is ≥100%.
Main results and the role of chance: The mean ± standard deviation (SD) age of the patients included was 38.7 (±5.2) years, the mean BMI was 25.8 (±5.5) kg/m^2, the mean basal FSH levels were 7.8 (±3.1) IU, the mean AMH levels were 4.5 (±6.1) ng/mL and the mean number of antral follicle count was 11.8 (±9.5). At OPU, the mean number (95% CI) of oocytes retrieved was 6.8 (6.1-7.5). In 299 out of 413 cycles (72.4%), hCG was used for triggering final oocyte maturation, whereas in 114 cycles (27.6%), this was performed with GnRH agonist. The smallest follicular diameter on the day of triggering final oocyte maturation with hCG, at which the lower 95% CI of oocyte retrieval rate was ≥100%, was 15 mm (mean oocyte retrieval rate: 133.7%, 95% CI: 109.8%-157.6%). The smallest follicular diameter on the day of triggering final oocyte maturation with GnRH agonist, at which the lower 95% CI of oocyte retrieval rate was ≥100%, was 16 mm (mean oocyte retrieval rate: 144.2%, 95% CI: 107.6%-180.8%).
Limitations, reasons for caution: The results obtained can be generalized only if similar practices to the current study are used for evaluating follicular development and performing oocyte retrieval. For instance, whether the results of the current study are also valid for cycles in which follicular flushing is performed needs to be assessed.
Wider implications of the findings: The findings of the present study allow for precise prediction of the number of oocyte retrieved depending on the triggering signal. In this way, patient consultation can be facilitated, especially in cases where an oocyte target has been set, such as preimplantation genetic testing or oocyte donation cycles.
Trial registration number: not required

P-609 Predictor for supraphysiologic serum estradiol elevation on hCG day of controlled ovarian stimulation (COS) using letrozole and gonadotropins in women with estrogen-dependent cancer

S.J. Han[1], M. Lee[1], S.W. Kim[1], H. Kim[1], J.Y. Lee[2], S.Y. Ku[1], C.S. Suh[1], S.H. Kim[1]

[1]Seoul National University Hospital, Obstetrics and Gynecology, Seoul, Korea- South ; [2]Hamchoon Women's Clinic, Obstetrics and gynecology, Seoul, Korea- South

Study question: Are there predicting factors for supraphysiologic estrogen elevation during COS with administration of letrozole in patients with estrogen-dependent cancer?
Summary answer: In the COS cycles using letrozole, early monitoring serum estradiol (E2) is an independent predicting factor for supraphysiologic level of serum E2 on hCG day.
What is known already: Supraphysiologic serum E2 levels (≥400 pg/ml) as a consequence of ovarian stimulation may promote the estrogen-dependent tumor growth or recurrence. Therefore, use of aromatase inhibitors is recommended to reduce the potential effects of elevated serum E2 levels in patients with estrogen-dependent cancers during ovarian stimulation. Previous studies that have shown an effective lowering of peak estrogen levels and feasible results of COS using the letrozole protocol have used a fixed dose of letrozole (5 mg/ day). However, a part of patients has supraphysiologic levels of estrogen associated with ovarian stimulation despite the administration of letrozole.
Study design, size, duration: From January 2009 to December 2019, patients with estrogen-dependent cancer who underwent COS with antagonist protocol using a fixed dose of letrozole (5 mg/ day) to keep estrogen levels low were included in this study. Cycles for fertility preservation and general in vitro fertilization cycles were all included. Subjects are consisted of 74 breast cancer patients, 5 endometrial cancer patients and 2 other estrogen-dependent cancer patients. A total of 94 COS cycles were retrospectively analyzed.
Participants/materials, setting, methods: Administration of letrozole was started on the second or third day of the menstrual cycle. Ovarian stimulation began with a dose of 150-450 IU/day on the same day or third day of letrozole administration and was continued until the hCG day. Dose of FSH was adjusted according to the patient's age, anti-Mullerian hormone (AMH), and body mass index (BMI). Early monitoring serum E2 was measured in all patients on the 4-6th day of stimulation.
Main results and the role of chance: Supraphysiologic levels of serum E2 was found in 20.2% of the patients who underwent COS with a fixed dose of letrozole (5 mg/ day) administration. Subjects were classified into two groups according to the serum E2 level on hCG day, physiologic E2 group (≤400 pg/ml) and supraphysiologic E2 group (>400 pg/ml). Mean age, BMI, AMH, basal serum LH/ FSH/ E2, total dose and duration of letrozole administered, duration of stimulation, total dose of gonadotropins administered were not different between the two groups. However, early monitoring serum E2 level was significantly higher in the supraphysiologic E2 group (68.9±49.3 vs. 119.8±78.1, p=0.001). Early monitoring serum E2 is associated with the occurrence of supraphysiologic elevation of serum E2 on hCG day (adjusted odds ratio [aOR] = 1.013; 95% confidence interval [CI], 1.003-1.022). The best cut-off for early monitoring serum E2 to predict the occurrence of supraphysiologic estrogen levels was 86.5 pg/ml (sensitivity was 73.7% and specificity was 73.3%). The mean area under a receiver operating characteristic (ROC) curve (AUC) was 0.731 (p=0.002 compared with 0.5).
Limitations, reasons for caution: Limitations of this study included the retrospective design and the small sample size. Prospective study will be needed to confirm that uptitration of letrozole is effective to prevent supraphysiologic levels of estrogen.
Wider implications of the findings: To prevent supraphysiologic elevation of serum E2, uptitration of letrozole should be considered when early monitoring serum E2 level is more than 86.5 pg/ml during COS with letrozole in patient with estrogen-dependent cancer.
Trial registration number: Not applicable

P-610 Severe OHSS after GnRH-agonist triggering is not eliminated if hCG is co-administered at triggering and/or during the luteal phase: a systematic review and meta-analysis

P. Ioannidou[1], J. Bosdou[1], G. Lainas[2], T. Lainas[2], N. Michaleli[1], G. Grimbizis[1], E. Kolibianakis[1]

[1]Aristotle University of Thessaloniki- Medical School, 1st Dept. of Ob/Gyn- Unit for Human Reproduction, Thessaloniki, Greece ; [2]Eugonia, Assisted Reproduction Unit, Athens, Greece

Study question: Is severe ovarian-hyperstimulation-syndrome (OHSS) after triggering with gonadotrophin-releasing-hormone agonist (GnRH-agonist) eliminated if human chorionic gonadotropin (hCG) is co-administered at triggering and/or during the luteal phase?
Summary answer: Severe OHSS, although low in incidence, is not eliminated, when hCG is administered either concomitantly with GnRH-agonist (dual-triggering) and/or during the luteal phase.

What is known already: Replacement of hCG by GnRH-agonist has decreased or even eliminated the incidence of severe OHSS. Concomitantly, however, the probability of pregnancy after fresh transfer and standard luteal phase support is decreased. This has been managed by freezing all embryos and deferring transfer to a future replacement cycle. Alternatively, in an effort to perform a fresh transfer, support of the luteal phase with hCG administered either concomitantly with GnRH-agonist (dual-triggering) and/or during the luteal phase has been advocated. The potential of both strategies, however, in eliminating severe OHSS has not been yet systematically assessed.
Study design, size, duration: Literature search was performed in MEDLINE and CENTRAL until 12/2019, in order to identify studies evaluating the incidence of severe OHSS following GnRH-agonist triggering in high-risk women. The incidence of severe OHSS was assessed separately for studies in which hCG was administered either concomitantly with GnRH-agonist(dual-triggering) and/or during the luteal phase, aiming to perform a fresh transfer and for studies in which no support was administered during the luteal phase and all embryos were frozen.
Participants/materials, setting, methods: Twenty-nine studies (n=2269 patients), published between 2000 and 2019, were included in this systematic review and meta-analysis. Pooling of severe OHSS incidence in the included studies is reported as a weighted average, by fitting the logistic regression model without covariate but an intercept, or by fitting the logistic-normal random-effects model without covariates but random intercepts. The confidence intervals are based on score (Wilson) or exact binomial (Clopper-Pearson) procedures.
Main results and the role of chance: Triggering of final oocyte maturation was performed with GnRH-agonist alone in 24 studies, whereas in five studies a combination of GnRH-agonist and hCG was used for this purpose.
The pooled mean number of oocytes retrieved in the high-risk for OHSS women included in these studies was 18.2 (95% CI: 15.0-21.5).

The pooled incidence of severe OHSS in high risk women triggered by GnRH-agonist who did not receive any form of luteal phase support was 0% (95% CI: 0.0-0.0, 15 studies, 1327 women).

The pooled incidence of severe OHSS in high risk women triggered by GnRH-agonist in whom hCG was added to standard luteal phase support was 0% (95% CI: 0.0-1.0, eight studies, 557 women).

The pooled incidence of severe OHSS in high risk women triggered by a combination of GnRH agonist and hCG (dual triggering), who received only estradiol and progesterone was 1% (95% CI: 0.0-3.0, three studies, 282 women).

The pooled incidence of severe OHSS in high risk women triggered by a combination of GnRH agonist and hCG (dual triggering) in whom hCG was in addition administered to standard luteal phase support was 1% (95% CI: 0.0-5.0, two studies, 103 women).
Limitations, reasons for caution: Despite the absence of statistical heterogeneity, clinical heterogeneity was present among studies regarding ovarian stimulation protocol, triggering signal, severe OHSS definition as well as the criteria used to define high risk probability for OHSS. Moreover, the number of patients/studies analysed in certain groups was limited.
Wider implications of the findings: Although the incidence of severe OHSS after GnRH-agonist triggering in high-risk women was low in all strategies examined, its elimination (upper 95% CI = 0) was only present in studies where no hCG was added. Thus, for elimination of severe OHSS, following GnRH-agonist triggering, addition of hCG is not recommended.
Trial registration number: not required

P-611 Cumulative live birth rates of oocyte in-vitro maturation and controlled ovarian stimulation in infertile patients with polycystic ovary syndrome

I. Segers[1], S. Santos-Ribeiro[1,2], L. Mostinckx[1], H. Tournaye[1], G. Verheyen[1], M. De Vos[1,3]

[1]UZ Brussel, Centre for Reproductive Medicine CRG, Jette- Brussels, Belgium ;
[2]IVI-RMA Lisboa, Reproductive medicine, Lisbon, Portugal ;
[3]Vrije Universiteit Brussel, Follicle Biology Laboratory, Jette-Brussels, Belgium

Study question: What is the clinical outcome of in-vitro maturation of oocytes (IVM) compared with controlled ovarian stimulation (COS) in polycystic ovary syndrome (PCOS) patients undergoing ICSI?
Summary answer: Overall, cumulative live birth rate (CLBR) was lower after IVM. However, in patients with hyperandrogenic PCOS phenotype A, CLBR after IVM and COS was similar.
What is known already: Published evidence suggests that embryo development and LBR are inferior following IVM compared to COS in patients with and without PCOS. Although PCOS phenotype has emerged as an important factor influencing COS and IVM treatment outcomes, assignment of PCOS phenotypes is lacking in patients enrolled in previous studies. PCOS women can be categorized into the following phenotypes: A= hyperandrogenism + ovulatory dysfunction + polycystic ovaries, B= hyperandrogenism + ovulatory dysfunction, C= hyperandrogenism + polycystic ovaries and D= ovulatory dysfunction + polycystic ovaries. Clinical outcomes of non-hCG triggered IVM and COS have not previously been compared among similar patient phenotypes.
Study design, size, duration: This was a retrospective, single-centre cohort study including 716 cycles in unique patients between 18 and 36 years of age who underwent either non-hCG triggered IVM (30h) after a short HP-hMG course (189 patients) or COS (527 patients) followed by ICSI and fresh or frozen embryo transfer between January 2010 and December 2016. First rank of trial were included.
Participants/materials, setting, methods: Predicted high responders with PCO-like morphology (PCOM) and/or levels of AMH ≥3.5 µg/l, or PCOS according to 2003 extended Rotterdam criteria, ≤37 years of age and scheduled for ICSI were included. Patient characteristics and IVM or COS treatment cycle data were collected. The primary objective was CLBR, defined as the rate of liveborns resulting from the transfer of all fresh and cryopreserved embryos from the same cycle. Secondary outcome was embryo quality.
Main results and the role of chance: IVM patients had higher BMI (24.8 vs 24.0 kg/m^2, p=0.03), higher basal antral follicle count (AFC (42.6 vs 26.6, p<0.001)), higher AMH (10.0 vs 6.4 µg/l, p<0.001) and were younger (28.3 vs 29.7y, p<0.001). Patients undergoing IVM had more often PCOS-A (19.7% vs 8.3%) and PCOS-D (65.4% vs 24.3%), a similar incidence of PCOS-C (4.3% vs 7.6%) and less often PCOM (10.3% vs 59.8%, overall p<0.001). IVM cycles yielded more oocytes (17.8 vs 13.9, p<0.001), lower maturation rates (51,7% vs 77.8%, p<0.001) and lower utilization rate per fertilized oocyte (51.0% vs 54.9%, p<0.05). After IVM, patients had more often transfer of vitrified/warmed cleavage stage embryos, whereas fresh transfer of blastocysts was more often performed following COS. Overall, CLBR was 40% after IVM and 59% after COS (p<0.001). There was no difference in CLBR between IVM and COS in patients with PCOS-A (43% vs 33%, p=0.45) whereas CLBR was higher (p<0.05) following COS compared to IVM in patients with PCOS-D (65% vs 41%) and PCOM (59% vs 30%).
Limitations, reasons for caution: This is a large observational study based on retrospective data collection. Despite our robust methodological approach, the presence of bias related to the retrospective design cannot be excluded. The low number of patients with PCOS-C impedes the validity of results in women with PCOS-C.
Wider implications of the findings: The current non-hCG triggered IVM system is an equally efficient mild-approach alternative for COS in patients with PCOS phenotype A. Further development of IVM systems could enhance the potential of IVM in a broader patient population.
Trial registration number: not applicable

P-612 The impact of endometrial thickness change between the day of ovum pick and embryo transfer on pregnancy outcome during in vitro fertilization cycle

M. Jeong[1], M.K. Chung[2], G.K. Lee[2], H.J. Jeong[1], H.S. Lee[1], M.H. Kim[1], J.W. Kim[1], E.B. Noe[1]

[1]Seoul Rachel Fertility Center, Medical Department, Seoul, Korea- South ;
[2]Seoul Rachel Fertility Center, Laboratory, Seoul, Korea- South

Study question: Is there any difference in pregnancy outcome according to the change of endometrial thickness in the patients undergoing in vitro fertilization-embryo transfer (IVF-ET) cycle?
Summary answer: Clinical and ongoing pregnancy rates were significantly higher when endometrial thickness did not increase on embryo transfer (ET) day compared with ovum pickup (OPU) day.
What is known already: Many studies have reported the importance of endometrial thickness on pregnancy outcome during in-vitro fertilization (IVF) cycle, most of them discovered that thin endometrium adversely affects pregnancy outcome. Previous studies have mostly measured the endometrial thickness on the human chorionic gonadotropin (hCG) trigger day in fresh IVF cycle

and examined its effect on pregnancy outcome. It is known that the endometrial condition is changeable not only in natural menstrual cycle but also in IVF cycle, yet few studies have examined the impact of endometrial thickness change within the IVF cycle.

Study design, size, duration: A retrospective study was conducted using data from a total of 397 fresh IVF cycles undertaken at a single fertility center. The study period went from January 2017 to November 2018. Only cases in which endometrial thickness on hCG trigger day, OPU day, ET day were all measured were included. Pre-implantation genetic test cycles or oocyte donation cycles were excluded.

Participants/materials, setting, methods: Each endometrial thickness of hCG trigger day, OPU day, and ET day were examined and its impact on pregnancy outcome was analyzed. Subsequently, endometrial changing pattern such as increase or decrease between the days were individually examined. Clinical and cycle characteristics, and pregnancy outcomes were compared according to the endometrial thickness change around OPU day.

Main results and the role of chance: The endometrial thickness of hCG trigger day or ET day was not significantly related to the pregnancy outcome. The endometrial thickness of OPU day was significantly higher in the implanted cycles than in the non-implanted cycles (9.29mm vs. 8.76mm, p <0.001), and showed the same tendency for cases with clinical pregnancy (9.28mm vs. 8.84mm, p=0.002) and ongoing pregnancy (9.40mm vs. 8.82mm, p <0.001). When analyzing the relationship between endometrial thickness changes and pregnancy outcome, only the impact of change between OPU and ET day on pregnancy outcome was identified. Baseline and cycle characteristics were not significantly different according to the changing pattern of endometrial thickness between OPU and ET day. Interestingly, the rate of implantation, clinical pregnancy and ongoing pregnancy were all significantly higher in the 'non-increased' group in which endometrial thickness were equal or decreased from OPU to ET day, compared with 'increased' group where endometrial thickness were thicker on ET day. After adjusting the transferred number of good quality embryos, clinical pregnancy (aOR=1.583, 95% CI: 1.018-2.464) and ongoing pregnancy (aOR=1.734, 95% CI: 1.101-2.728) rates were still significantly higher in the 'non-increased' group than in the 'increased' group.

Limitations, reasons for caution: Limitations of this study included the retrospective design and the heterogeneity of the subjects, including such as infertility diagnosis or ovarian stimulation method which may affect the pregnancy outcome in IVF cycle. Therefore, prospective studies are needed before generalizing the results.

Wider implications of the findings: Though we have generally measured endometrial thickness on hCG day and considered that thickness to be crucial factor for IVF outcome, this study result suggest that endometrial thickness on OPU day and its change until ET day may be more important clues for predicting pregnancy outcome.

Trial registration number: not applicable

P-613 Genomics analysis of maternal exomes reveals new candidate genes and pathways for the diagnosis and prediction of recurrent preimplantation embryo arrest in IVF cycles

S. Kahraman[1], F. Sagir[1], B. Yuksel[1], M. Cetinkaya[1], M. Gavaz[1], B. Kara[1], M. Yesil[1], G. Akar[1], S. Buonaiuto[2], G. Damaggio[2], M. Fabiani[3], A. De Marino[4], V. Colonna[2], C. Simon[5], A. Capalbo[4]

[1]Istanbul Memorial Hospital-, ART and Reproductive Genetics Center, Istanbul, Turkey ;
[2]National Research Council CNR, Institute of Genetics and Biophysics, Naples, Italy ;
[3]Igenomix Italy, Genetics and Biophysic, Marostica, Italy ;
[4]Igenomix Italy, Genetics and Biophysics, Marostica, Italy ;
[5]Valencia University and INCLIVA, Department of Obstetrics and Gynecology, Valencia, Spain

Study question: Is genomic analysis of maternal exomes capable of identifying new target genes to improve infertility diagnosis and support the development of new treatment strategies?

Summary answer: Exome analysis of woman with recurrent embryo arrest successfully identify genomic variants lethal at the embryonic stage increasing diagnostic yield toward unexplained infertility

What is known already: In IVF cycles, it is estimated that about 40% of the human oocytes are capable of growing to the blastocyst stage, whereas others arrest at different early stages of development. It is commonly thought that much of human embryonic developmental potential is determined before fertilization by the content of the oocyte (maternal-effect genes). Infertile patients commonly show individual variability in embryo developmental rate, ranging from high to total developmental failure in multiple treatment cycles. The genetic determinant of this phenotype is unknown and this study aims at exploring the genetic aetiology of extreme phenotypes of preimplantation embryonic arrest

Study design, size, duration: Ten women (five with consanguinity history, four being the offspring of first-cousin marriage) with a history of recurrent embryo developmental failure in multiple IVF cycles were recruited at Istanbul Memorial Hospital (Dec 2018-Nov 2019). These were all young women (average age 32.3 y), with good ovarian reserve (average AMH 3.6) and with several failed IVF cycles (average 4.4, range 3-8), following the retrieval of high number of mature oocytes per procedure (average 11.2).

Participants/materials, setting, methods: Whole-exome sequence was performed by Agilent SureSelect whole-exome capture and Illumina sequencing technology. Variant calling against the reference genome GRCh38 was done using Freebayes. Variants were annotated with a custom script that integrates information from Ensembl99 with publicly available manually curated lists of genes associated with embryonic development, miscarriages, lethality, cell cycle. The code is available on gitHub. The Hospital Ethical Committee approved the study and data were treated anonymously.

Main results and the role of chance: Variant calling identified on average 436k high quality variants per samples. According to Ensembl classification 2.8% are expected to have high (0.25%) or moderate (2.56%) disruptive impact in the gene product. Variants were filtered on a per-individual base using three criteria. First, alleles with frequency >0.05% in the 1000 Genomes and gnomeAD reference populations were filtered out. Second, variants were ranked by severity (as estimated by Ensembl) according to their Sequence Ontology classification, and only variant in the last percentile were retained. Finally, variants were assigned a score that correlates with the gene relevance in early embryonic development and only variants in the last fifth percentile were retained.

Overall, 62 unique variants in 54 genes were retained after filtering, all involved in lethal embryonic pathways. All retained variants have high impact and half of them is a stop gain. Each sample carry on average 8.6 (2.7 s.d.) potentially detrimental variants. Of particular relevance seven samples share stop gain and splice donor mutations at four sites in three exones of the MTCH2 gene, a nuclear-encoded transporters localized in the inner mitochondrial membrane, involved in the energy and lipid metabolism, never reported associated to preimplantation embryo arrest.

Limitations, reasons for caution: Functional genomics studies and validation in an independent cohort of patients with preimplantation embryo arrest phenotype and of different ethnicity is required to corroborate these findings. The generation of polygenic models will also further contribute increasing discovery rate and to develop more general and powerful predictive models for this phenotype.

Wider implications of the findings: We have identified MTCH2 and several new candidate genes as the plausible cause of female infertility characterized by early embryonic arrest. Such discoveries not only contribute to the recognition of novel gene function/pathways in reproduction but also help the development of genetic diagnosis and suggest possible therapeutic targets for infertility.

Trial registration number: 25

P-614 NLRP3 inflammasome analysis in grannulose-cumulus cells from low ovarian reserve patients

C. Andres[1], L. Rancan[2], C. Cordero[1], R. Pandolfi[3], M. Saladino[3], C. Rodriguez[1], B. Linillos[4], V. Elena[2], J. Tresguerres[4], C. Susana[1]

[1]Clinica tambre, IVF Laboratory, Madrid, Spain ;
[2]Complutense University, Biochemistry and molecular biology, Madrid, Spain ;
[3]Clinica tambre, Andrology Laboratory, Madrid, Spain ;
[4]Complutense University, Physiology Department, Madrid, Spain

Study question: Is the inflammasome NLRP3, together with other inflammatory markers, activated on low ovarian reserve patients and is this correlated with the number of follicles retrieved?

Summary answer: Inflammasome activation was observed in patients with low ovarian reserve (LOR) in comparison to donors with normal response. It is not correlated with follicle number.

What is known already: LOR patients are women, within the reproductive age group, which are characterized by having a decreased ovarian follicular pool. They present abnormal but not post-menopausal ovarian reserve test results. There is a wide-range of origins that can lead to this pathology.

Oxidative stress associated with chronic low-grade inflammation has emerged as a plausible key contributor to the pathogenesis. The NLRP3 inflammasome can start an inflammatory form of cell death and trigger the release of proinflammatory cytokines through caspase activation. Although it has been associated to different diseases, an association with low ovarian reserve has not been described to date.

Study design, size, duration: Prospective clinical observational study conducted at Clinica Tambre in collaboration with the University Complutense of Madrid. A total of 16 donors and 9 patients participated in the study. Stimulation protocols were the same for all patients (FSHr and triggering with GnRH analogues). Collection of oocyte-granulose complexes (GCs) was performed during oocyte retrieval. GCs were processed according to the procedure described by Ferrero F, et al. 2012 throughout 2019. All patients signed the specific informed constent.

Participants/materials, setting, methods: mRNA expression of NLRP3, IL-1β, IL-1 receptor, caspase 1, 12 and 18, and serpin were analyzed in the CGs complexes as well as the number of follicles retrieved to evaluate correlation with gene expression. mRNA was measured by qRT-PCR. The 2-ΔΔCT method was used for relative gene expression changes calculation. Non parametric tests were used to identify significant differences between groups and spearmen correlation to asses correlation.

Main results and the role of chance: Increased mRNA expression was observed in LOR patients compared to donors (p=0.022). Accordingly, LOR patients had increased expression of IL-1B and its corresponding receptor in comparison to donors (p=0.0003 and p=0.0001 respectively). In the same manner, caspase 1, 12 and 18 were also significantly increased in our group of patients in comparison to the donor expression (p<0.0001, p=0.0008 and p=0.0003 respectively). Finally, pathway's inhibitor expression: SERPIN B9 was significantly higher in donors (p=0.0002).

When analyzing the correlation with the oocytes retrieved, we observed no significant correlation between the number of oocytes obtained with the expression of NLRP3 and Serpin (p= 0.2977 and p=0.7158, respectively). In contrast, we did observe a negative significant strong correlation in inflammation markers IL-18 (p=0,0069, r -0,6583) , IL-1B (p=0,0009, r-0,8244) and IL-1R (p=0,0009, r-0,824) and a significant negative mild correlation in caspase 1 (p=0,0102, r-0,5357) and 12 (p=0,0488, r-0,5616).

Our results show higher NLRP3 expression in patients with low ovarian reserve compared to donors suggesting an activation of the pathway and its possible involvement in the pathogenesis. Additionally, there seems to be a negative correlation in between the number of oocytes retrieved and inflammatory markers.

Limitations, reasons for caution: Due to the low number of patients who participated in our study, in order to confirm our results, further analysis is required.

Wider implications of the findings: Finding out the causes behind idiopathic LOR will give us a hint for a better approach to the treatment or ways to improve IVF outcomes.

Trial registration number: Not aplicable

P-615 Diabetic but not women with normal metabolic phenotype with unexplained infertility are in risk for decreased ovarian response : A prospesctive population based cohort study

S. Kalapahar[1], S. Sharma[1], R. Chattopadhyay[1], S. Parvin[1], S. Behera[1], S. Ghosh[1], P. Chakraborty[1], B. Chakravorty[1]

[1]Institute of Reproductive Medicine, Assisted Reproductive Medicine, KOLKATA, India

Study question: Does diabetes confer a differential ovarian response and IVF outcome through diminished connexin-43 expression in women with unexplained infertility(UI) undergoing ovarian stimulation?

Summary answer: Ovarian reserve and ovarian response to stimulation is lower in diabetic patient along with decreased connexin-43 expressions in comparison to non-diabetic women with unexplained infertility.

What is known already: Recent studies cue at the association of diminished ovarian reserve with diabetes mellitus. Reduced ovarian reserve in diabetes might be associated with decreased oocyte developmental competence leading to poor IVF outcome. Attenuation of ovarian gap junction protein (connexin-43) involves decreased follicular development and oocyte growth retardation. However, there is little information whether diabetes targets connexin-43 to alter follicular growth. Anti –mullerian hormone(AMH) and Antral follicular count(AFC) are the most important factors predicting ovarian stimulation response based on retrieved oocyte number. Hence, we have tried to find out the effect of diabetes on ovarian stimulation response in these women undergoing IVF.

Study design, size, duration: This is a prospective cohort study in women (n=415) with UI in age group between 25 and 35 years, conducted between August 2017 and December 2019 at Institute of Reproductive Medicine,Kolkata. Patients have been divided to diabetic(n=216) controlled with either tablet Metformin or insulin and non-diabetic based on ADA guidelines. All patients are stimulated using recombinant FSH with individualized dosing and GnRH antagonist (flexible)protocol .Fresh or frozen embryo transfer has been done within 1 year.

Participants/materials, setting, methods: All patients have been evaluated for HbA1C, AMH & AFC. All diabetic patient either taking tablet Metformin and/or insulin with HbA1C <7 have been included in the study group. After ovum pickup, follicular fluid of each patient has been evaluated by immunoblotting for connexin-43 expression in between granulosa cells. Routinely 2 blastocyst have been transferred.

Two tailed Student's *t*- test is used to evaluate significance of differences.p value <0.05 is considered to be significant.

Main results and the role of chance: Baseline parameters (Age,obesity) are comparable between two groups .AMH (1.69 ± 0.9 vs 2.97±0.3, p<0.05) & AFC (8±1.9 vs 10.4±2, p<0.05) are significantly less in diabetic patient than non-diabetic group indicating low ovarian reserve.Total gonadotropin dose requirement is significantly higher in diabetic group (2873±75.08 vs 2497±80.15, p<0.05). Oocyte recovery /patient is also lower, but not significant (7±1.5 vs 9±1.7). M II oocyte percentage is significantly lower in diabetic group (73±2.1 vs 80±1.5, p<0.05). Connexin-43 expression is also significantly lower in diabetic group obtained by immunoblot technique. Fertilization rate (62.16±5.7 vs 73.5±2.9), cleavage rate (87.7±6.1 vs 91.8±5.3) and blastocyst development rates (58.6±3.9 vs 65.9±6.2) are non-significantly less in diabetic group. But clinical pregnancy rate is significantly low (56.7 vs 64.1, p<0.05) in diabetic group after blastocyst transfer either in fresh or frozen cycle. Duration of diabetes has negative correlation (- 0.403) with number of M II oocyte retieved.

Limitations, reasons for caution: It is an observational study with limited number of patients. Exact cause or mechanism of decreased response is not revealed by this study. Before wide application of this observation, other undetected cause of diminished ovarian response should be searched for and study with larger sample size is warranted.

Wider implications of the findings: Diabetic women with infertility should be counseled regarding low ovarian reserve and decreased ovarian response irrespective of age. Diabetic women are associated with increased gonadotropin requirement and poor IVF outcome.

Trial registration number: not applicable

P-616 Can we detect a biologically relevant quantity of Anti-Mullerian Hormone (AMH) in human hair samples?

S. Sawarkar[1], M.Á. Rodríguez[2], L. Garcia-Calvo[2], A. Carbajal[2], B. Ring[1], Y.C. Vives[3], M.H. Duvison[4], A. Crisci[1], C.O. Marieta[5], M. Jimenez[6], S. Munne[1], M. Bejar[2]

[1]MedAnswers Inc., Research Development & Analytics, San Pedro, U.S.A. ;
[2]Universitat Autònoma de Barcelona, Dpt. Animal Health and Anatomy Veterinary Faculty, Barcelona, Spain ;
[3]Complejo hospitalario Ruber Juan Bravo, Unidad de Reproducción Asistida, Madrid, Spain ;
[4]Ginemed, Reproduccion asistida en Clinicas, Seville, Spain ;
[5]CER Santander, Medicina de la reproducción, Cantabria, Spain ;
[6]Sinae, Research, Seville, Spain

Study question: Can we detect a biologically relevant quantity of **Anti-Mullerian Hormone (AMH)** in human hair samples?

Summary answer: AMH can be detected in human hair samples, and levels of AMH in hair are correlative to maternal age.
What is known already: AMH is a product of granulosa cells of the preantral and small antral follicles in women. Hence, AMH is often used as a biomarker in assessing fertility. Typically, circulating levels of AMH are tested using blood samples obtained invasively through median cubital vein punctures. Hormone concentrations in hair may serve as a comparable and possibly superior means of assaying hormone levels accrued over longer periods of time. Detection of steroid hormones in hair has been used in psychoneuroendocrinological studies in human and companion, farm and wild animals. This study represents the first quantification of AMH levels in hair in humans.
Study design, size, duration: The study design was prospective in nature. A total of **(n=152)** human female participants between the ages of **18-65 years** were included in the study over a period of 10 months (recruitment ongoing).
Participants/materials, setting, methods: Sample collection was performed in a clinical setting. Blood and hair samples were collected from patients by nurses. Hair follicles are not required. A doctor or a clinical technician performed the ultrasound for measuring the antral follicle count (AFC). Biologically active AMH was extracted from hair using a proprietary method. AMH presence in hair extract was confirmed using Western Blotting. AMH was measured in plasma and serum by ELISA.
Main results and the role of chance: AMH was successfully detected in samples (n=152) via western blots on denatured gel with bands at 70kDa. An average level of **9.37 pg/ml (95%CI 6.77-12)** was detected in hair and **3.68 ng/ml (95%CI 2.79-4.56)** in serum in **age-group <25 yrs.** This is in contrast to the age group **>39 years**, within which a mean of **3.02 pg/ml (95%CI 2.19-3.85)** AMH detected in hair and **0.92 ng/ml (95%CI 0.43-1.41)** in serum samples. AMH measured in hair correlated with age more strongly than plasma AMH **(p-value =1.26 x10-5 (hair), p-value 0.088 (serum)**). AMH levels in hair also strongly correlated with antral follicle count (AFC).
Limitations, reasons for caution: Hair is a medium that can accumulate biomarkers over several weeks, while serum is an acute matrix representing only current levels. Range of detection of AMH in hair was wide within individuals from a similar age cohort. AFC testing was included in the study laterally and has limited data points.
Wider implications of the findings: We have a novel method of detecting AMH in a longitudinal matrix (hair) that could be a more appropriate representation of hormone levels compared to acute matrices like serum or saliva. Moreover, our method is also the only truly non-invasive method for testing fertility hormones.
Trial registration number: Not Applicable

P-617 Endocrine disruptors in the serum and follicular fluid of a cohort of Italian women undergoing Assisted Reproduction Techniques

L. De Santis[1,10], F. Pallotti[2,10], A.P. Dima[2,10], R. Ciriminna[3,10], R. Gualtieri[4,10], V. Pisaturo[5,10], M.G. Minasi[6,10], L.F. Rienzi[7,10], C. Scarica[8,10], A. Lenzi[2,10], F. Botrè[9,10], D. Paoli[2,10], On behalf of SIERR(10)[segreteria@sierr.it]

[1]*San Raffaele Scientific Institute, Ob/Gyn IVF UNIT, Milan, Italy ;*
[2]*"Sapienza" University of Rome, Laboratory of Seminology- Sperm Bank "Loredana Gandini", Rome, Italy ;*
[3]*Centro AMBRA, Fertility Unit, Palermo, Italy ;*
[4]*University of Naples "Federico II", Department of Biology, Naples, Italy ;*
[5]*International Evangelical Hospital, Reproductive Medicine, Genoa, Italy ;*
[6]*European Hospital, Centre for Reproductive Medicine, Rome, Italy ;*
[7]*Laboratory GENERA, Centres for Reproductive Medicine, Rome, Italy ;*
[8]*Casa di cura Villa Salaria in Partnership with Institut Marques, Centres for Reproductive Medicine, Rome, Italy ;*
[9]*"Sapienza" University of Rome, Department of Experimental Medicine, Rome, Italy ;*
[10]*SIERR, Italian Society of Embryology Reproduction and Research, Italy*

Study question: Are human oocytes exposed to common plastic Endocrine Disruptors (EDs) present in the follicular fluid (FF) and blood serum (BS) before retrieval?
Summary answer: Monobutyl-phthalate (MBP), monoethylhexyl-phthalate (MEHP) and Bisphenol A (BPA) were detected in FF and BS and associated to geographical area, source of exposure and clinical features.
What is known already: Folliculogenesis is a strictly regulated process, requiring interactions of androgenic, estrogenic and gonadotropin signalling. EDs can affect this process and, thus, reproduction by creating hormonal imbalances through different and sometimes not well-established molecular mechanisms. Few *in vitro* evidences have associated several phthalates esters and BPA to antral follicle growth inhibition. Urinary EDs are generally considered markers of exposure, but they may not reflect accurately in vivo exposure of oocytes.
Study design, size, duration: A multicentre study involving six fertility centres across Italy (Milan, Turin, Rome, Naples, Bari, Catania) was conducted between 2018 and 2019, aiming to prospectively recruit at least 120 women attending fertility treatments. EDs testing was carried out in a centralised Laboratory (Seminology Laboratory "*Loredana Gandini*"- "Sapienza", University of Rome) with a standardized collection protocol and a unique disposable material provider.
Participants/materials, setting, methods: The designated sites recruited 122 women undergoing ART living in their respective geographical area for at least 3 years, aged ≤42 years, with normal ovarian reserve (AMH≥1 ng/ml and FSH≤12 IU/L) and no active ovarian pathologies including PCOS. Subjects completed a questionnaire investigating potential sources of contamination. Blood and Follicular Fluid samples were taken for EDs measurement (MBP, MBzP, MEHP, MEHHP, MEOXP and BPA) using liquid chromatography tandem mass spectrometry (LC-MS/MS).
Main results and the role of chance: The main EDs found in BS and FF were MBP (median BS: 8.96 ng/ml, FF 6.43 ng/ml), MEHP (median BS: 9.16 ng/ml, FF 7.68 ng/ml) and BPA (median BS: 1.89 ng/ml, FF 1.86 ng/ml) which were above the limit of detection in both biological matrices in 97%, 77% and 25.4% of subjects, respectively. A significant correlation between serum and follicular concentration was present for MBP and BPA (Spearman's ρ: MBP 0.567, p<0.001; BPA 0.682, p<0.001). Multivariate analyses showed a significant interaction between serum and follicular MBP from the geographical area and the daily use of plastic food packaging (PFP) (p<0.001, partial η2=0.093). In particular, post-hoc univariate analyses detected that serum MBP concentration was significantly associated with geographical area (p<0.001, adj. mean 13.56 ng/ml, 14.40 ng/ml, 7.61 ng/ml, South, Centre and North Italy respectively) and negatively with home PFP (p=0.004), suggesting that different sources of exposure than PFP may impact more on serum MBP levels. Moreover, follicular but not serum MBP was associated with irregular cycles prior controlled ovarian stimulation (p=0.019). No association was detected between EDs and eating habits, living in the area of hazardous sites, work contact with potential toxicants and other clinical features.
Limitations, reasons for caution: Despite several potential environmental sources of EDs contamination, only a few of them were associated to EDs concentration in both serum and follicular fluid, suggesting other routes of exposure and justifying the need for more prospective investigations in larger sample size.
Wider implications of the findings: This study represents the first Italian biomonitoring of plastic EDs in FF. The finding of MBP and MEHP in follicular fluid confirms the few previous reports available from other countries. This investigation may prove to be the basis for future prospective evaluation to correlate follicular EDs concentration and oocyte quality.
Trial registration number: not applicable

P-618 IL-6/sIL-6R Increases COX-2 Expression and PGE2 Production in a Human Granulosa-Lutein SVOG Cell Line Via a JAK2/STAT3/SOCS3 Signaling Pathway

L. Saijiao[1], H.M. Chang[2], J.H. Wang[2], J. Yang[1], P.C.K. Leung[2]

[1]*Renmin Hospital of Wuhan University, Department of Reproductive Medicine Center, Wuhan, China ;*
[2]*BC Children's Hospital Research Institute- University of British Columbia-, Department of Obstetrics and Gynaecology, Vancouver, Canada*

Study question: The functional role of IL-6 in the regulation of ovarian Prostaglandin E2 (PGE_2) in human granulosa cells is controversial and the detailed molecular mechanisms remain to be elucidated
Summary answer: The IL-6 trans-signaling-induced activation of JAK2/STAT3/SOCS3 and subsequently increase COX-2 expression and PGE_2 production in hGL cells.
What is known already: As a critical paracrine regulator of multiple reproductive functions, the cytokine interleukin-6 (IL-6) is expressed in human

granulosa cells and detected in follicular fluid. At present, the functional role of IL-6 in the regulation of ovarian Prostaglandin E2 (PGE_2) is controversial. Moreover, the detailed molecular mechanisms by which IL-6 regulates the production of PGE_2 in human granulosa cells remain to be elucidated. Up to now, no reports were related to the effect of the IL-6 trans-signaling pathway on PGE2 production and need further investigation.

Study design, size, duration: Primary human granulosa-lutein (hGL) cells and a non-tumorigenic immortalized human granulosa-lutein cell line, SVOG, were used for this study. Dose, time-course, inhibitors, and siRNA were performed in this experiment.

Participants/materials, setting, methods: Primary human granulosa-lutein (hGL) cells and a non-tumorigenic immortalized human granulosa-lutein cell line, SVOG, were used for this study. The quantitative PCR and westerns blot were used to measure the mRNA, proteins, and phosphorylation of proteins expression; immunofluorescence staining was used to measure the translocation of STAT3; the small interfering RNA transfection was used to knockdown the STAT3 and SOCS3 RNA, and ELISA was used to measure the prostaglandin E2 production.

Main results and the role of chance: The IL-6 trans-signaling using the combined addition of IL-6 and soluble IL-6 receptor (sIL-6R) induced COX-2 expression and PGE_2 production in hGL cells. Additionally, IL-6/sIL-6R activated the phosphorylation of Janus activated kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3), induced the STAT3 nuclear translocation, which was inhibited by inhibitors AG490 (JAK2 inhibitor) and C188-9 (STAT3 inhibitor), as well as siRNA-mediated knockdown of STAT3. Moreover, the IL-6 trans-signaling-induced activation of JAK2/STAT3 unregulated the expression of suppressor of cytokine signaling 3 (SOCS3), which, in turn, negatively regulated the JAK/STAT3 signaling pathway by suppressing the activity of STAT3 and the subsequent COX-2 expression and PGE_2 production in hGL cells.

Limitations, reasons for caution: we did not study the interacting effect of IL-6/sIL-6R with gonadotropins because these hGL cells have been exposed to the high doses of gonadotropins during the clinical treatment cycles, which cause the cellular expression levels of gonadotropin receptors and the cellular response to gonadotropin could be relatively low.

Wider implications of the findings: Our findings shed light on the cellular and molecular mechanisms by which the IL-6/sIL-6R-induced JAK2/STAT3/SOCS3 signaling pathway modulates the synthesis of PGE2 in the human ovary.

Trial registration number: The Canadian Institutes of Health Research Foundation Scheme Grant (#143317); the National Natural Science Foundation of China Grant (# 81701412); the Nature Science Foundation of Hubei Province Grant (# 2018CFB491)

P-619 Pregnancy outcomes in Turner syndrome

E. Burt[1], M. Davies[1], E. Yasmin[1], A. Cameron- Pimblett[1], G. Conway[1]

[1]University College London Hospital, Reproductive Medicine Unit, London, United Kingdom

Study question: Assessing pregnancy outcomes in women with Turner syndrome (TS)

Summary answer: Initial results show an overall high miscarriage rate, no acute cardiovascular morbidity and a 16% vertical transmission of TS.

What is known already: Turner Syndrome (TS) affects 1:2500 females and is caused by the partial or complete loss of one X chromosome. About 80% of women with TS experience primary amenorrhea and therefore the only option for fertility treatment is ovum donation (OD). The remaining 20% may have the opportunity for a spontaneous pregnancy. Pregnancy in women with TS has been associated with excess obstetric risk such as miscarriage and hypertension. Estimates for maternal mortality have varied between 0 and 2% in TS mainly due to the risk of aortic dissection but until now there was no UK data.

Study design, size, duration: Retrospective single centre cross-sectional study

Participants/materials, setting, methods: Over 850 women with TS have attended the adult TS clinic at University College London Hospital and those who had achieved pregnancy were identified. Telephone interviews were conducted to collect data regarding; mode of conception, mode of delivery, TS-specific complication such as cardiac events and hypertension and neonatal outcomes.

Main results and the role of chance: Seventy-nine women with TS had a total 136 pregnancies of which 37% were spontaneous conceptions and 63% were achieved with ovum donation (OD). The live birth rate was 58.1% and miscarriage rate 36%, and this was comparable in spontaneous and ovum donation pregnancies. There were no significant differences in the prevalence of gestational hypertension, preeclampsia and gestational diabetes between the two groups. No case of acute cardiovascular morbidity such as aortic dissection has been identified. There were 5 cases (16%) of vertical transmission of TS to daughters in those with spontaneous conception.

Limitations, reasons for caution: This is a retrospective study reliant on participant recall.

Wider implications of the findings: The higher rate of miscarriage has been previously documented but the comparable rates between those undergoing OD and spontaneous conception is reassuring. Whilst maternal cardiac morbidity has been reported, in our cohort there were no acute events identified. The 16% vertical transmission exceeds that previously recorded in the literature.

Trial registration number: n/a

P-620 miR-424 suppresses the proliferation and promotes the apoptosis of human ovarian granulosa cells by targeting Apelin and APJ expression

J. Du[1], X. Lin[1], R. Wu[1], Z. Gao[2], Y. Du[1], Y. Liao[1], J. Wu[1], W. Ke[1], S. Quan[3]

[1]Boai Hospital of Zhongshan, Reproductive Medical Center, Zhongshan, China ; [2]Boai Hospital of Zhongshan, Department of Gynecology, Zhongshan, China ; [3]Nanfang Hospital- Southern Medical University, Reproductive Medical Center-Department of Obstetrics and Gynecology, Guangzhou, China

Study question: This study aims to investigated the roles of miR-424 in modulating Apelin expression and GC functions of Polycystic ovary syndrome (PCOS) .

Summary answer: miR-424 suppresses the proliferation and promotes the apoptosis of human ovarian granulosa cells by directly targeting and inhibiting Apelin and APJ expression.

What is known already: Polycystic ovary syndrome (PCOS) development is mediated by alteration of Apelin signaling in ovarian granulosa cells (GCs).

Study design, size, duration: We aimed to investigate the roles of miR-424 in regulating Apelin and APJ receptor expression in the context of PCOS, as well as their involvement in granulosa cell proliferation and apoptosis.The follicular fluid and serum samples used for analysis were collected from patients with PCOS who were registered at the Reproductive Medical Center of Boai Hospital of Zhongshan between Jun 1st, 2015 and Dec 31st, 2018. Healthy volunteers were included as the control group.

Participants/materials, setting, methods: miRNA expression in GCs were altered by transfection with specific mimics and inhibitors. Apelin concentration was determined by ELISA, and gene and miR-424 expression were analyzed by quantitative RT-PCR. Protein abundances were measured by western blotting. Association of miR-424 with genomic sequences were validated by dual-luciferase reporter assay. Apelin gene expression was promoted by LV-003 plasmid-mediated cell transfection. GC proliferation was analyzed by MTS method, and its apoptosis were measured by flow cytometry.

Main results and the role of chance: Apelin concentration was increased in serum and follicular fluid from PCOS patients, accompanied with accelerated APJ (Apelin receptor) expression and suppressed miR-424 expression in GCs. miR-424 mimics suppressed Apelin and APJ expression in KGN cells by targeting Apelin and APJ 3'-UTR regions, which was recovered by miR-424 inhibitors. miR-424 inhibited KGN cell proliferation and cell cycle progression by down-regulating Cyclin-D/E expression. Moreover, miR-424 promoted KGN cell apoptosis by elevating truncated Caspase-3 level. The regulation of KGN cell proliferation and apoptosis by miR-424 was mediated by directly suppressing Apelin gene expression, but not the inhibition of Apelin peptide activity.

Limitations, reasons for caution: We use luteinized granulosa cells and KGN cell lines, so whether there is the same effect on non luteinized granulosa cells needs to be discussed.

Wider implications of the findings: We first revealed that miR-424 expression was greatly down-regulated in the ovarian granulosa cells from PCOS

patients, but Apelin and APJ expression showed opposite alterations, miR-424 could directly target the 3'UTR regions of both the Apelin and APJ gene in KGN cell line.

Trial registration number: not applicable

P-621 PRP in recurrent implantation failure, hope or hype !? A Prospective randomized controlled study

K.A. Ibrahim Rageh[1], A. Barakat[1], A. Mohamed[2]

[1]Al-Baraka fertility hospital, Ivf, Manama, Bahrain ;
[2]Al-Baraka fertility hospital, IVF lab, Manama, Bahrain

Study question: PRP in recurrent implantation failure, hope or hype !?

- We designed this study to evaluate the effectiveness of endometrial perfusion of PRP in improvement of pregnancy rate in RIF patients

Summary answer: The PRP significantly improved the pregnancy rate and may be a new hope for management of recurrent implantation failure; which is one of the nightmares

What is known already: The endometrial receptivity have been accepted to be major limiting factors in the establishment of pregnancy. In spite of improved almost all aspects of IVF: ovarian stimulation, embryo culture and transfer, the pregnancy rates still not satisfactory. The bottleneck is the process of implantation.

Recurrent implantation failure (RIF) is one of the nightmares in reproductive medicine and despite several strategies that have been described for management; there is no universal agreement yet.

Recently, PRPis described to promote endometrial growth and receptivity, PRP has been investigated as a therapeutic approach for several medical disorders, but its use in IVF is still limited.

Study design, size, duration: Study design;
prospective randomized controlled study
sample size ;
150 participants
duration;
from July 2018 to March 2019.

Participants/materials, setting, methods: 150 infertile women with history of RIF gave their consent to be included in this study- with age below 40 yrs, body mass index (BMI) below 30 kg/m2- from July 2018 to March 2019.Divided into 2 comparable groups; all underwent antagonist protocol, In the study group, intrauterine infusion of 0.5 to 1 ml of PRP was performed 48 hrs before blastocyst transfer, pregnancy tests were done 12 days after ET.

Main results and the role of chance: The result of our study revealed that endometrial perfusion of platelet-rich plasma (PRP) significantly improved the pregnancy rate and may be a new hope inthose patents with a history of recurrent implantation failure (RIF). Out of 75 participants in each group, 32 got pregnant (43%) in the study group after endometrial PRP infusion, compared to 11 pregnant participants (15%) in the control group.

The main strengths of our study include: (1) this is by far the first study discussing this topic in our country, as our hospital is the leader IVF center here in Bahrain. (2)The low risk of bias between study and control group as both of them were statistically comparable. (3) We exclude those patients with hematological and immunological disorders, hormonal disorders, chromosomal and genetic abnormalities and uterine abnormalities (acquired or congenital) as confirmed by HSG and U/S to limit additional factors that may affect the results of the study. (4) All Blastocyst transfers were performed under ultrasound guidance by only one expert gynecologist with infertility fellowship. (5) The use of individual patient data for direct comparison between both groups. (6) The consistency between our results and those of the previous trials in the literature.

Limitations, reasons for caution: as we are living in small country so, sample size may need to be increased in the further coming clinical trials, also at time of ET we select them phenotypically according to the embryo scoring, not based on genetic basis as we are usually not doing PGT as a routine;

Wider implications of the findings: RIF is hard to be managed, and if all available treatments fail, then PRP can add value. PRP that contains several growth factors and cytokines may improve endometrial receptivity and implantation. PRP is collected from autologous blood sample, so in comparison to G-CSF, PRP is more accessible and affordable

Trial registration number: NCT04085783

P-622 The entire range of trigger-day endometrial thickness values in fresh in vitro fertilization cycles is in direct independent correlation with the live birth rate

Y. Shufaro[1], M. Simeonov[2], Y. Lande[2], A. Ben-Haroush[3], G. Oron[3], E. Shlush[2], E. Altman[3], A. Wertheimer[2], T. Shochat[4], O. Sapir[3]

[1]Beilinson Women's Hospital- Rabin Medical Center- and the Felsenstein MRC- Tel-Aviv University, Infertility and IVF Unit, Petach-Tikva, Israel ;
[2]Beilinson Women's Hospital- Rabin Medical Center, Infertility and IVF Unit, Petach-Tikva, Israel ;
[3]Beilinson Women's Hospital- Rabin Medical Center- Tel-Aviv University, Infertility and IVF Unit, Petach-Tikva, Israel ;
[4]Beilinson Women's Hospital- Rabin Medical Center, Research Authority, Petach-Tikva, Israel

Study question: What is the association of the entire range of the trigger-day endometrial thickness (EMT) with the live birth rate (LBR) following in vitro fertilization (IVF) and fresh embryo-transfer (ET) ?

Summary answer: The entire range of EMT is in direct correlation with LBR in IVF-fresh-ET cycles, even after adjusting for age and ovarian response to stimulation.

What is known already: The association between EMT and LBR in IVF cycles with fresh ET is unclear. Some studies reported a positive association of EMT with pregnancy rates but in other studies such a correlation did not exist. There are also conflicting reports regarding the impact of an increased EMT (≥ 13 mm) on treatment outcome.

Study design, size, duration: A cohort study of all IVF cycles with fresh day-3 ET in patients age ≤42 in a single centre. LBR was calculated for all EMT values, stratified into 5 groups (≤6,7-9,10-12,13-15,and ≥16 mm), overall and within subgroups of patient age (≤35,36-40,41-42 years) and ovarian response(1-7,8-15,>16oocytes). Univariate analysis and multivariate logistic regression model were used for adjusting for various independent variables. General linear models were generated to compare continuous variables between EMT, age and oocyte groups.

Participants/materials, setting, methods: 5133 IVF cycles, performed in 2343 female patients age 42 or younger, in a single center between 2009-2017 were included. Fresh ET was performed on day 3 in all included cycles.

Main results and the role of chance: LBRs were as follows: 11.22% (35/312) in cycles with EMT ≤6mm, 17.98% (280/2114) in cycles with EMT 7-9mm, 23.44% (476/2031) in cycles with EMT 10-12mm, 25.62% (144/562) in cycles with EMT 13-15mm, and 34.21% (39/114) in cycles with EMT ≥16 mm (P <0.001). Similar findings were observed on further sub-group analysis according to patient age and ovarian response. The entire range of trigger-day EMT values in fresh cycles was found to be in direct correlation with the LBR, even after adjusting for confounders of age and ovarian response to stimulation. This observation was confirmed also by a multivariate logistic regression analysis in which the EMT was found to be a significant independent predictor of LBR controlling for various confounders. Excessively thick endometrium (≥13mm) was not detrimental, but rather beneficial, for treatment outcome.

Limitations, reasons for caution: Retrospective study, proving that EMT is an important independent prognostic factor for a favourable ART outcome. The findings of the study are relevant only for cycles in which fresh ET is performed. This study does not address the question if interventions to increase the EMT are beneficial.

Wider implications of the findings: The pre-trigger EMT is in significant independent correlation with the live birth rate. We suggest that stimulation cycles should be planned and managed to achieve maximal endometrial proliferation, and that fresh ET can be performed at high EMT values without endangering the outcome of the cycle.

Trial registration number: NA

P-623 Aberrant BMP15/HIF-1α/SCF signaling pathway in human granulosa cells is involved in the PCOS related abnormal follicular development

J. Tan[1]

[1]Jiangxi Provincial Maternal and Child Health Hospital, Reproductive Medicine Center, Nanchang, China

Study question: To explore the related regulatory mechanisms of stem cell factor (SCF) expression in PCOS related abnormal follicular development.

Summary answer: BMP15 induce SCF expression by up-regulating HIF-1α in human GCs, aberrance of this signaling pathway might be involved in PCOS related abnormal follicular development.
What is known already: Our previous studies have found that stem cell factor (SCF), which is an important granulosa cells (GCs) derived growth factor in follicular development, is significantly reduced in PCOS related abnormal follicles. However, the related regulator mechanisms of SCF expression in PCOS are still uncompleted clear. The present study is to further investigate the regulatory mechanism of SCF expression in human GCs, which can help us to better understand the abnormal development of PCOS related follicles
Study design, size, duration: The present study includes human serums, follicular fluids (FFs) and GCs were collected from 69 PCOS patients and 74 normal ovulatory patients during the IVF therapy, respectively. Human granulosa cell line (KGN) was also used in this study.
Participants/materials, setting, methods: ELISA was used to evaluate the concentrations of SCF and BMP15 in FFs and serums. KGN cells were treated with recombinant human BMP15 alone or in combination with BMP type I receptor inhibitors and transfected with hypoxia inducible factor-1α siRNA before detecting the target genes and proteins by RT-PCR and Western blot, respectively. The rates of metaphase II, oocyte, fertilization, embryo cleavage and good quality embryo between PCOS group and non-PCOS group were analyzed.
Main results and the role of chance: The results showed that the rate of MII oocyte and 2PN fertilization was significantly lower in PCOS group than those in non-PCOS group, though PCOS patients retrieved much more oocytes. No difference was observed for the cleavage rate and high-quality embryo rate between these two groups. Furthermore, the concentration of BMP15 in FF and the concentration of SCF in serum and FF were also significantly lower in PCOS patients. Interestingly, we found a weakened expression of HIF-1α and SCF in GCs from PCOS patients when compared with the non-PCOS patients. The expression of HIF-1α and SCF was significantly increased in KGN cells after treating cells with rhBMP15, However, this promotion effects of BMP15 on HIF-1α and SCF expression were obviously abolished by co-treatment with DM. Moreover, knock down of HIF-1α expression in KGN cells by transfecting cells with HIF-1α siRNA significantly reduced the expression of SCF in human GCs, in spite of activating BMP15 signaling pathway.
Limitations, reasons for caution: The present results were obtained from human GCs and KGN cell lines, which might not be fully representative of the true human PCOS environment.
Wider implications of the findings: This study showed a significantly reduction of BMP15, HIF-1α and SCF in PCOS related abnormal follicles, and demonstrated that BMP15 could induce SCF expression by up-regulating HIF-1α expression in human GCs. The aberrance of this signaling pathway might be involved in the PCOS related abnormal follicular development.
Trial registration number: not applicable

P-624 The ovarian yield – number of oocytes per antral follicular count (AFC) score and anti-Mullerian hormone (AMH) levels – does not predict ART outcome.

"Abstract withdrawn by the authors"

P-625 Hyperandrogenism regulates adipocytes expandasion and differentiation in polycystic ovary syndrome

Y. Qian[1], D. Yang[1]

[1]Sun Yat-sen Memorial Hospital, Reproductive medicine center, Guangzhou, China

Study question: What is the effect of androgen excess on adipocyte differentiation in PCOS?
Summary answer: Androgens, via the androgen receptors, exert direct effects on adipocyte differentiation and may leads to the metabolic disorder in PCOS women.
What is known already: Polycystic ovary syndrome (PCOS) women are at risk for developing metabolic syndrome, impaired glucose tolerance and cardiovascular disease. Androgen excess is a common feature among women with PCOS and has been suggested as associated with adipose tissue disturbance. We hypothesized that androgens play an important role in regulation of adipogenesis and adipocyte functions including lipid metabolism in PCOS.
Study design, size, duration: 17 women with PCOS and 24 normal women who underwent laparoscopic operation due to tubal factors were recruited. We collected omental adipose tissue samples (approximately 1 cm^3size) during the surgery and analyzed gene expression level.
Participants/materials, setting, methods: Primary human preadipocytes, isolated from omental adipose tissue, were cultured in vitro. Dihydrotestosterone-treated cells were used as study models. The regulation of adipogenesis was confirmed by Western blot and Oil Red O staining after androgen stimulation.
Main results and the role of chance: PCOS women had increased serum concentrations of testosterone (P = 0.001) and higher HOMA-IR levels (P = 0.04), with increased expression of androgen receptor (AR) in adipose tissue. In vitro studies indicated that androgens, via the AR, enhanced lipogenesis in human primary preadipocytes. DHT-treated cells produced more lipid droplets on Day8 in vitro. Dose-response experiments performed in human preadipocyte cultures showed that expression of adipogenic transcription factors CCAAT/enhancer-binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ) increased after androgen exposure.
Limitations, reasons for caution: The mechanism of adipocyte dysfunction and metabolic disorder mediated by androgen in PCOS should be further evaluated.
Wider implications of the findings: Our results suggested that adipose tissue was an important site linking androgen and metabolism in PCOS. PCOS is an androgen excess disorder associated with adipose tissue disturbances. For obese patients with hyperandrogenism, exercise accompanied with anti-androgenic therapy could be efficient than those only accept lifestyle interventions to achieve weight loss.
Trial registration number: not applicable

P-626 Vitamin D supplementation prior to initiate IVF: a randomized controlled study

M. Reschini[1], V. Sarais[2], S. Ferrari[1], G.C. Cermisoni[2], A. Paffoni[3], S. Signorelli[2], E. Papaleo[2], E. Somigliana[1], P. Viganò[2]

[1]Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Infertility Unit, Milano, Italy ;
[2]IRCCS San Raffaele, Centro Scienze Natalità, Milan, Italy ;
[3]ASST Lariana, Infertility Unit, Cantù, Italy

Study question: Does vitamin D supplementation improve pregnancy rate in women undergoing IVF?
Summary answer: In IVF cycles, Vitamin D supplementation does not increase the chances of pregnancy.
What is known already: Vitamin D plays an important role in human physiology and pathology and vitamin D insufficiency has been shown to be associated with several diseases. There is also growing evidence supporting a role of vitamin D in reproductive health, especially in female fertility. In particular, IVF success was shown to be higher in women with appropriate reserves of vitamin D. However, a causal relation has not been established and evidence from RCTs exploring the possible benefits of vitamin D supplementation is sparse, under-powered and inconsistent.
Study design, size, duration: A multicenter randomized double blinded placebo controlled study was conducted to investigate the possible benefits of vitamin D supplementation. Women referring for IVF in two ART Centres from October 2018 to January 2019 were considered for study entry. The main inclusion criterion was a serum vitamin D level <30 ng/ml. Eligible women received either Vitamin D (600.000 IU) or placebo in a single oral administration between 2 and 12 weeks before to oocyte retrival.
Participants/materials, setting, methods: Inclusion criteria of patients were: indication to IVF, age 18-39 years, ≤2 previous oocytes retrievals and BMI between 18 and 25 kg/m^2. Poor responders according to Bologna criteria were excluded as well as patients using surgically-retrieved spermatozoa. Both participants and physicians were blinded to the allocation. The main outcome was cumulative pregnancy rate per retrieval. The analysis was by intention to treat.
Main results and the role of chance: Overall, 738 patients were initially selected. Eighty-eight (12%) were subsequently excluded because their serum

vitamin D was ≥30 ng/ml and 20 (3%) declined participation after being initially selected. The remaining 630 women were randomized, 308 received Vitamin D and 322 were treated with placebo. 21 (7%) and 22 (7%) dropped-out prior to initiate the cycle, respectively. The total number of women obtaining a clinical pregnancy in treated and control women was 123 (40%) and 131 (41%), respectively (p=0.85). Women achieving ongoing pregnancies / live births were 100 (32%) and 111 (34%), respectively (p=0.59). The Relative Risk (RR) of clinical and ongoing pregnancy / live births were 0.98 (95%CI: 0.81 – 1.19, p=0.85) and 0.94 (95%CI: 0.76 – 1.17, p=0.59), respectively. When focusing on IVF cycles characteristics, no main differences emerged. In particular, duration of hyperstimulation (p=0.91), total dosage of gonadotropins administered (p=0.98), number of retrieved oocytes (p=0.22), IU of gonadotropins per retrieved oocyte (p=0.55) and fertilization rate (p=0.33) did not differ.

Limitations, reasons for caution: The mode of administration of vitamin D may be inappropriate for improving IVF outcomes. One may claim that an earlier initiation of the supplementation (5-6 months prior to initiate the cycle) and a daily administration could be effective.

Wider implications of the findings: The routine administration of vitamin D prior to initiate IVF in order to increase the probability of pregnancy is not recommended.

Trial registration number: EUDRACT 2015-004233-27

P-627 Impact of polyunsaturated fatty acid supplementation on assisted reproductive technology (ART) outcomes: a systematic review

E. Bayar[1], S. Saso[1], N. Galazis[2], B. Jones[1], T. Bracewell-Milnes[3], M. Chawla[4], Y. Ahmed-Salim[1], J. Ben Nagi[5]

[1]Imperial College NHS Trust, Obstetrics & Gynaecology, London, United Kingdom ;
[2]Northwick Park NHS Trust, Obstetrics & Gynaecology, London, United Kingdom ;
[3]Chelsea and Westminster NHS Hospital, Obstetrics & Gynaecology, London, United Kingdom ;
[4]North Middlesex University Hospital, Obstetrics & Gynaecology, London, United Kingdom ;
[5]The Centre for Reproductive and Genetic Health, Fertility, London, United Kingdom

Study question: This review explores the role of dietary polyunsaturated fatty acid (PUFA) supplementation in women undergoing assisted reproduction technology (ART) in order to guide recommendations for clinical practice.

Summary answer: There is a need for greater quality randomised control trials (RCTs) to facilitate an in depth understanding of how PUFA supplementation may impact ART success.

What is known already: Modifiable risk factors are important targets for improving reproductive health and often represent the first interventional step in maximising the chances of conception. Over recent years, diet and nutritional supplementations have received increasing attention. Women undergoing ART are frequently targeted with readily available over the counter supplementations in an attempt to boost conception chances. Fatty acids play an important role in basic cellular function and animal studies have suggested a beneficial role on oocyte quality and embryogenesis. This has led to the suggestion that PUFA supplementation may improve reproductive outcomes in women undergoing ART.

Study design, size, duration: The primary outcomes assessed included pregnancy, live birth, implantation and miscarriage rates. PRISMA guidelines were used to conduct this review. An electronic database search was performed using MEDLINE, EMBASE and the Cochrane Library to identify articles published from January 1978 to 2019. Abstracts were screened for suitability following which, full text articles were reviewed in detail. Additional studies identified through screening review articles were also considered.

Participants/materials, setting, methods: A total of 102 studies were identified through searching of databases. A further 12 studies were retrieved through manual searching and review papers. 114 papers were screened, with 90 exclusions, leaving 24 studies for full-text review. Of these, nine studies were then excluded (five papers did not address the proposed research question, three were review articles and one study was on-going). Overall 15 studies were eligible for inclusion in our systematic review.

Main results and the role of chance: In total, 15 studies met the inclusion criteria for this review, four of which were RCTs. Although the overall aim was shared between the included RCTs, meta-analysis could not be performed due to disparities between intervention including dose, content, population characteristics and measured outcomes.

Whilst most authors focus on identifying whether a health benefit of PUFA supplementation exists, potential risks must also be explored. None of the included studies explored miscarriage rates, congenital anomalies or obstetric outcomes such as birth weight and prematurity.

Importantly, a complex link between genetic variation and lipid metabolism exists, thus challenging the comparison between studies, which included different demographic groups. Gene-diet interactions must be considered but are difficult to evaluate.

Overall, there were distinct differences between population groups, study design and measured outcomes. Studies showing a benefit were of low quality and only six of the included studies assessed live birth rates as an outcome.

Limitations, reasons for caution: The main limitation was the disparity between study methodologies. Assessment of PUFA intake varied between studies; some assessed dietary intake via validated food questionnaires whilst others measured serum fatty acid or follicular fluid fatty acid concentrations. Comparison between studies was also hindered by different ART protocols used in institutions.

Wider implications of the findings: Although the ovarian micro-environment is an important contributor to conception and embryo development, there is a lack of high quality research to support dietary PUFA supplementation in women undergoing ART. In conclusion,a need exists for well-designed RCTs to facilitate the understanding of PUFA supplementation in women undergoing ART.

Trial registration number: N/A

P-628 A typical temperature patterns as an aid to identify infertility issues and miscarriage risk

M. Karoshi[1], B. Hurst, S[2], R. Milnes[3], T. Knowles[4], A. Pirrie[5]

[1]Royal Free Hospital and UCL Medical School, Gynaecology, London, United Kingdom ;
[2]Carolinas Medical Center, Department of Assisted Reproduction, Charlotte, U.S.A. ;
[3]Fertility Focus Limited, Research and Development, Warwick, United Kingdom ;
[4]University of Bristol, Faculty of Medical and Veterinary Sciences, Bristol, United Kingdom ;
[5]Fertility Focus Limited, Research & Development, Warwick, United Kingdom

Study question: To determine if ovulatory abnormalities and risk of miscarriage were associated with atypical Patterns of vaginal core body temperature (CBT) measurements from the OvuSense system.

Summary answer: Identified atypical OvuSense (OS) CBT Patterns are more likely to occur with "infertility related diagnoses" including PCOS, PCOS with regular cycles, and elevated miscarriage risk.

What is known already: Three novel, atypical CBT Patterns published previously, confirmed in updated population of 18,679 ovulatory cycles: (a) "Crash To Baseline" = first nightly averaged CBT falls by >0.2 degrees Celsius (^{0}C) to lowest cycle CBT point (baseline), (b) "False Start" = rise of >0.1^{0}C did not result in ovulation but instead a return to baseline CBT followed by ovulation two or more days later in the cycle, (c) "Crash After Ovulation" = final CBT >0.2^{0}C lower than the post ovulatory peak CBT. It is likely OS patterns closely reflect progesterone changes, hence cycle-related hormonal abnormalities may be associated with atypical patterns.

Study design, size, duration: Retrospective, longitudinal, comparative, observational study. Participants used OS vaginally at night to monitor CBT when not menstruating. The total study population (TSP) was 18,679 ovulatory cycles from 8,653 OS users recorded between March 2016 and December 2019. A detailed medical questionnaire was then issued to study participants and the answers from 375 respondents accounting for 1,491 of the TSP cycles was used for further assessment.

Participants/materials, setting, methods: TSP used to confirm prevalence of cycle Patterns (a)-(c); and questionnaire assessed per respondent for each following existing 'Diagnosis':

1. Any infertility related diagnosis
2. PCOS
3. PCOS and regular cycles
4. Previous miscarriage = gravida >0, number miscarriages >0.

Odds Ratio (OR) calculated as (a/b)/(c/d) for each Pattern + Diagnosis combination together with their 95% confidence interval: a. Positive Diagnosis (*+D*), Pattern >1 cycle for respondent (*+P*); b. *-D+P; c. +D-P; d. -D-P.*

Main results and the role of chance: The prevalence of each Diagnosis in the total respondent population of 375 was: 217 (57.9%) with 1. Any infertility related diagnosis; 156 (41.6%) with 2. PCOS; 53 (14.1%) with 3. PCOS and regular cycles (i.e. 34.0% of those with Diagnosis of PCOS report regular cycles); 145 (38.7%) with 4. Previous miscarriage. Note: respondents can potentially have more than one Diagnosis and Pattern.

(a) "Crash To Baseline" 14.0% of TSP, and 263 cycles from 164 respondents. OR: 1.44 for 1 (CI 0.95-2.18); 1.69 for 2 (1.12-2.56); 15.71 for 3 (7.90-31.23); and 6.17 for 4 (3.59-10.63).
(b) "False Start" 9.1% of TSP, and 202 cycles from 133 respondents. OR: 1.69 for 1 (CI 1.12-2.56); 2.84 for 2 (1.83-4.39); 9.52 for 3 (4.80-18.87); and 6.99 for 4 (3.89-12.57).
(c) "Crash After Ovulation" 11.4% of TSP, and 216 cycles from 128 respondents. OR: 1.10 for 1 (CI 0.71-1.70); 1.61 for 2 (1.04-2.47); 1.45 for 3 (0.80-2.62); and 7.04 for 4 (3.82-12.99).

These results indicate strong associations between reported Diagnosis and the atypical CBT Patterns identified using the OS system.

The high OR for each Pattern associated with miscarriage merits further investigation, as the cohort has a low pregnancy rate.

Limitations, reasons for caution: The authors note the co-existence of Pattern + Diagnosis is not strictly "predictive", as each Diagnosis is by definition historic. The population is by definition biased to one or more Diagnosis as over 57% of respondents report having been trying to conceive for a year or more prior to use.

Wider implications of the findings: Results suggest that atypical CBT Patterns may aid diagnosis, and in particular elevated risk of miscarriage. It should be noted that the absence of an existing Diagnosis does not necessarily render the results with positive Patterns "false", and the existence of a Pattern could anyway indicate investigation for ovulatory abnormalities.

Trial registration number: Atrium Health IRB File #03-19-16E

P-629 The Effect of Cushing's syndrome on Pregnancy Complication Rates: Analysis of More than 9 Million Deliveries

H. Baghlaf[1], A. Badeghiesh[2], E. Suarthana[2], M. Dahan[2]

[1]Toronto, Obstetrics and Gynecology, Toronto, Canada ;
[2]McGill University, Obstetrics and Gynecology, Montreal, Canada

Study question: What are maternal and fetal morbidities that increase with Cushing's syndrome (CS)?

Summary answer: CS increases risks of pre-eclampsia, blood transfusion, and operative vaginal deliveries, but unexpectedly not preterm deliveries and gestational diabetes compared to the control group.

What is known already: Cushing's syndrome (CS) rarely complicates pregnancy. This may be due to the induced infertility via alterations in gonadotropins and androgen. CS is believed to increases maternal and fetal morbidities, including preeclampsia, gestational diabetes, preterm delivery and intrauterine growth restriction. This knowledge is based solely on case reports and small case series. A recent systematic review and meta-analysis confirmed the above findings and it included only case reports and case series. Studies with control groups are lacking in the literature and this study was conducted to compare pregnancies complicated with CS to pregnancies in general population

Study design, size, duration: We conducted a retrospective population-based cohort study utilizing data from the Health Care Cost and Utilization Project-Nationwide Inpatient Sample database (HCUP-NIS) over 11 years from 2004 to 2014. We created a cohort of all deliveries between 2004 and 2014 inclusively. Within this group, all deliveries to women with CS were identified as part of the study group (n=135), and the remaining deliveries were categorized as non-CS births and comprised the reference group (n=9,096,653).

Participants/materials, setting, methods: Analysis was performed to identify the prevalence of pregnant women with CS over the study duration. Demographic and clinical characteristics were compared between women with and without CS using Chi-square test. All confounding variables were adjusted for using multivariate logistic regression, based on any significant differences between the two groups generating adjusted odds ratios (aOR).

Main results and the role of chance: We identified 9,096,788 pregnancies during the study period. CS complicated 135 pregnancies at a rate of 1-2 cases per 100,000 births. CS subjects were more likely to be older (p<0.001), obese (p<0.001), have private insurance (p<0.001), chronic hypertension (24.4% versus 1.8%, p<0.001), and pre-gestational diabetes (7.4% versus 0.9%, p<0.001). The maternal mortality rate was 0.7% and 0.007% in CS and control groups, respectively. Preeclampsia was higher in CS compared to controls after controlling for baseline risk factors, including pre-existing hypertension (aOR 2.20, 95% CI 1.18-4.41). Operative vaginal delivery and blood transfusion rates were high in CS compared to controls after controlling for confounding factors, aOR 6.49 (95% CI 4.50-9.38) and aOR 3.09 (95% CI 1.35-7.07), respectively. The rates of preterm delivery (8.9% versus 7.2%) and gestational diabetes (8.1% versus 5.8%) were not statistically different between CS and control groups, aOR 0.82 (95% CI 0.45-1.56) and aOR 0.82 (95% CI 0.44-1.56), respectively.

Limitations, reasons for caution: This retrospective analysis utilizes an administrative database, with its inherent limitations. Significant medical history or adverse pregnancy outcomes may be more often reported in patients with more significant conditions or outcomes

Wider implications of the findings: CS patients often begin pregnancies with maladies making them at risk for complications. These patients might benefit from prevention methods for preeclampsia, and increased surveillance to decrease maternal morbidity and mortality. Certain aflictions which were increased in case series were not found to be elevated in CS in this study

Trial registration number: not applicable

P-630 Early pregnancy loss in patients with polycystic ovary syndrome: in vitro maturation of oocytes versus controlled ovarian stimulation

L. Mostinckx[1], S. Mackens[1], P. Drakopoulos[1], I. Segers[1], S. Santos-Ribeiro[2], B. Popovic-Todorovic[1], H. Tournaye[1], C. Blockeel[1], M. De Vos[1]

[1]Universitair Ziekenhuis Brussel, Centre for Reproductive Medicine, Brussels, Belgium ;
[2]IVI-RMA Lisboa, Gynecology/Reproductive Medicine, Lisbon, Portugal

Study question: Is early pregnancy loss (EPL) increased in PCOS patients undergoing ICSI following in-vitro maturation of oocytes (IVM) when compared with controlled ovarian stimulation (COS)?

Summary answer: PCOS patients who achieved a pregnancy following IVM-ICSI were more at risk for EPL compared to their counterparts who were pregnant after COS-ICSI.

What is known already: Although some studies have reported comparable miscarriage rates after IVM and COS in women with PCOS, others have reported increased miscarriage rates after IVM. Nevertheless, apart from their limited sample size, many of these studies were performed using hCG-triggered IVM protocols, and patient or treatment characteristics that may contribute to EPL were not considered. Therefore, we aimed to compare EPL after non-hCG triggered IVM and COS following fresh or frozen embryo transfer in patients with a specific phenotypical diagnosis of PCOS.

Study design, size, duration: This was a retrospective, single-centre cohort study including 800 pregnant infertile women between 18 and 36 years of age with PCOS as defined by the extended Rotterdam criteria who underwent either IVM or COS between January 2010 and December 2017.

Participants/materials, setting, methods: Non-hCG triggered IVM (28-40h) after a short HP-hMG course, or conventional COS, followed by ICSI and fresh or frozen embryo transfer (ET). Only the outcome of the first ET resulting in a biochemical pregnancy was included in the analysis. The main outcome measure was EPL (biochemical pregnancy loss (BPL) + miscarriage) before ten weeks' gestation. Relevant patient characteristics were analysed in order to develop a multivariate logistic model for independent risk factors of EPL.

Main results and the role of chance: Outcomes in 329 IVM patients were compared with those of 471 COS patients. Rank of ET was comparable (0.84 ± 1.23 vs. 1.04 ± 1.64 previous ETs respectively in the IVM and COS groups). Pregnant IVM patients had higher AMH levels (11.5 ± 8.1 ng/mL vs. 7.2 ± 4.1 ng/mL, p< 0.001) compared to pregnant COS patients. Hyperandrogenic PCOS

phenotypes were more common among IVM patients (59.9% vs. 48.2%, p= 0.001). Women who were pregnant after COS had previously suffered EPL more often compared to IVM patients (28% vs. 17.6%, p= 0.003). Pregnancies after IVM were more often obtained after frozen ET (62.9%) compared to those after COS (35.2%, p< 0.001). Transfer of a cleavage stage embryo was more common in pregnant IVM patients (62.6%) compared to COS patients (32.1%, p< 0.001). The BPL rate (13.1% after IVM vs. 9.3% after COS, p=0.09) was similar, but IVM patients had a higher miscarriage rate before ten weeks' gestation (23.4% vs. 14.6%, p=0.002). In a multivariate logistic model allowing adjustment for relevant confounders, IVM was the only independent factor (adjusted OR 1.62, 95% CI 1.12-2.33) associated with increased odds of EPL.

Limitations, reasons for caution: This is a large observational study based on retrospective data collection. Despite our robust methodological approach, the presence of bias related to the retrospective design cannot be excluded.

Wider implications of the findings: The observation of an increased risk of EPL following IVM warrants further research that should result in improved IVM laboratory protocols conducive to enhanced embryo quality and improved developmental potential.

Trial registration number: not applicable

P-631 Association of ovarian stimulation with embryonic aneuploidy in in vitro fertilization (IVF) cycles: a narrative systematic review

J. Rodriguez-Purata[1], M.J. Gomez-Cuesta[1], E. Cervantes-Bravo[1]

[1]CdelaF, Clinica de la Fertilidad, Cuajimalpa de Morelos, Mexico

Study question: Is there an association of controlled ovarian stimulation (COS) with embryonic aneuploidy in IVF cycles coupled with preimplantation genetic testing (PGT)?

Summary answer: Although even natural cycles are associated with aneuploidy, more robust stimulations are associated with aneuploidy. Nevertheless, a higher response is associated with more euploid embryos.

What is known already: According to recent literature, the impact of COS on the rate of embryo aneuploidy in patients without the negative effect of age acting as a confounding factor is still a subject of lively debate. Moreover, embryo aneuploidy is present even in unstimulated cycles, suggesting that, either this is the real incidence in human beings, or there are factors other than COS related with the IVF procedure which may increase this incidence in comparison with in vivo fertilization. A systematic review of the available evidence is needed to provide a succinct analysis of the most relevant findings to date.

Study design, size, duration: The study adhered to the PRISMA guidelines. A systematic search for studies was performed at MEDLINE, ClinicalTrials.gov, PubMed, Embase, CINAHL and Cochrane Library. . Searches were coordinated by an expert librarian and a statistician in March'19. Search updates were conducted in July'19. The PICO model was used to select the study population. The search retrieved 73 citations, and 15 were eligible for analysis (4805 cycles). Average quality Newcastle-Ottawa scale (NOS) score was 8.

Participants/materials, setting, methods: Women/couples who underwent COS for an IVF cycle to genetically analyze her/their embryos through PGT were included. There were no restrictions in the amplification method, or the platform used to analyze the amplified DNA. Included studies were additionally subclassified according to the protocol of stimulation. Within subgroups, special attention was put to oocytes retrieved, average and total number of euploid embryos, and proportion of euploid/aneuploid embryos. When possible, an intention-to-treat (ITT) analysis was performed.

Main results and the role of chance: A clear direct correlation between COS and embryonic aneuploidy was not found. Studies have exposed that absence of COS does not rule out the occurrence of aneuploidy. It is important to consider that when only analyzing patients that reach biopsy-stage or had at least one embryo biopsied, we are excluding patients who are at the higher risk of producing an aneuploid embryo: older patients or with low-quality oocytes/embryos. Although there is evidence to believe that fewer oocytes retrieved render better quality under similar dose, more robust studies have proved that aneuploidy rate did not increase with ovarian response or gonadotropin dosage. Particularly, no differences were found when using an agonist or antagonist protocol. Nevertheless, FSH-only stimulations influenced aneuploidy rates under a long-agonist, but not under an antagonist protocol. Trigger medication (hCG vs GnRH-agonist) was not predictive of aneuploidy. Among the studies associating ovarian response to aneuploidy, only Haaf et al reported an increased aneuploidy rate, although statistical limitations were observed. All other 6 studies reported similar results, concluding that ovarian response did not alter the euploidy rate or the total number of euploid embryos. Moreover, a case can be made towards better results with an increasing number of biopsied embryos.

Limitations, reasons for caution: Only one RCT has been performed. Even though the quality of the rest of the studies is high, bias associated to retrospective studies cannot be ruled-out. Studies presented a large diversity in the assessment of embryonic aneuploidy, and, given the great heterogeneity between studies, a meta-analysis could not be performed.

Wider implications of the findings: Due to the fact that the number of euploid embryos available for transfer increases as the number of oocytes obtained does, considering the absolute number of euploid embryos seems more relevant than the proportion of euploid/aneuploid embryos, especially when cumulative LBR outcome is ascertained as the optimal outcome.

Trial registration number: CRD42019120803

P-632 Poor ovarian reserve and response are associated with interleukin 10 levels in women undergoing in-vitro fertilization

A.M. Fabregat Reolid[1], M. Hortal[1], B. Lledó[1], J.A. Ortiz[1], A. Bernabeu[2], J. Llácer[2], R. Bernabeu[2]

[1]Instituto Bernabeu, Biotech, Alicante, Spain ;
[2]Instituto Bernabeu, Reproductive Medicine, Alicante, Spain

Study question: Are cytokines related to ovarian reserve and response in ART?

Summary answer: Interleukin-10 is strongly and positively related to ovarian reserve and response parameters, providing a potential new tool in the prediction of controlled ovarian stimulation outcome

What is known already: Previous studies reported differences in the levels of IL-2, Il-6, IL-8, IL-10 and VEGF in follicular fluid between young patients with low ovarian response and normoresponder women. Cytokine levels have also been analyzed in women with low ovarian reserve who have Hodgkin lymphoma, obtaining a negative correlation between AMH, SIL-2R, IL-6 and IL-8 levels. Although there seems to be some evidence about the possible effect of the immune system on ovarian function and implantation, the role it plays in the success of ART remains unknown. Our aim was to investigate the effect of cytokines in ovarian reserve and response

Study design, size, duration: One hundred and twenty-six patients were included in a retrospective study between February 2016 and November 2018. Cytokines IL-2, IL-4, IL- 6, IL-8, IL-10, VEGF, IFNγ, TNFα, IL-1α, IL-1β, MCP-1 and EGF were measured previously to the ovarian stimulation cycle. Patients with abnormal results of karyotype and/or FMR1 gene or with any other factor that could alter the ovarian reserve or response were excluded from the study

Participants/materials, setting, methods: To measure the levels of the different cytokines, a sandwich immunoassay with specific antibodies for the cytokines IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNγ, TNFα, IL -1α, IL-1β, MCP-1 and EGF were used. The statistical analysis was performed with the Software Statistical Product and Service Solutions, version 20.0 (SPSS, Chicago, IL, EE.UU.)

Main results and the role of chance: We found that the most related cytokine with the different ovarian reserve and response markers is interleukin-10. It significantly correlates with AMH (p=0.011), antral follicle count (p<0.001), total oocytes (p<0.001) and MII retrieved (p=0.019). Statistically significant relationships had also been found between antral follicle count and IFN- γ (p=0.036), total oocytes with IL-6 and TNF- α (p=0.012 in both cases) and MII retrieved with IL-6 and TNF- α (p=0.043 and p=0.014, respectively). Through multivariate analysis, we obtained a model able to predict 49.9% of the variability in the number of total oocytes recovered using only 3 variables: age, antral follicle count and interleukin 10 levels. Statistical differences were observed when we compare patients with interleukin 10 below or above the threshold of 0.5 pg/ml in the mean number of total oocytes (6.52 versus 11.05; p<0.001) and MII retrieved (4.45 versus 8.05; p<0.001). Moreover, in women with interleukin 10 below 0.5 pg/ml, the dose of gonadotropins and the number of total oocytes recovered is not associated (p=0.495). Therefore, a higher dose of gonadotropins is not related to a better ovarian response in these patients

Limitations, reasons for caution: The retrospective study design and the sample size could be a limitation. The study was performed in patients with suspected implantation failure or endometriosis. Despite the strong relationship

of interleukin 10 with ovarian reserve and response, we cannot determine its role in the ovary, beyond its immunomodulatory function
Wider implications of the findings: Interleukin 10 level is lower in patients with low ovarian reserve and response compared to normoresponder patients. Moreover, if the interleukin 10 level is below 0.5 pg/ml, tailoring the treatment might not improve the outcome. In conclusion, interleukin 10 could be used as a biomarker to predict the ovarian response
Trial registration number: Not applicable

P-633 Could follitropin delta individualized dose, reduce the overall ovarian hyperstimulation syndrome risk (OHSS)?

A. VAZQUEZ SARANDESES[1], L. De la Fuente Bitaine[1], L. Marqueta Marquez[2], M. Abad[1], M. Carrera Roig[1]

[1]Hospital Universitario 12 de Octubre, reproductive medicine, madrid, Spain ; [2]Hopital Universitario 12 de Octubre, Reproductive Medicine, Madrid, Spain

Study question: Does the individualization of the starting dose of follitropin delta reduce the OHSS risk in selected patients at risk of high response to stimulation
Summary answer: Individualized dosing with follitropin delta (d-FSH) significantly reduced the risk of OHSS, allows more women to undergo fresh embryo and those embryos had better quality
What is known already: OHSS is an iatrogenic and potentially life-threatening complication of ovarian stimulation. It mostly occurs in patients at risk of presenting a high response to stimulation, *"high responders"*: antral follicle count (AFC), high serum levels of anti-müllerian hormone (AMH) or number of oocytes retrieved greater than 15. Managing each woman's risk of OHSS while maximizing her potential for a successful outcome constitutes one of the major clinical challenges in IVF treatment. Delta follitropin is administered according to an individualized dosing algorithm based on serum AMH levels and body weight, that seeks to administrate the most accurate dose to each patient.
Study design, size, duration: January 2017-December 2019, we included women aged 18–40 with regular ovulatory cycles and diagnosed with tubal infertility, unexplained infertility, endometriosis stages I/II or with partners with male factor infertility, that underwent a previous cycle using recombinant follicle-stimulating hormone(rFSH) and met one of the criteria for high responders. We performed a study with intrasubject measurements, to evaluate the changes in the response after each controlled ovarian stimulation(COS):1stcycle with rFSH Vs. 2ndcycle with d-FSH
Participants/materials, setting, methods: We collected data following a pre-post design and we compared the differences between each patient both cycles. T-student and χ^2 test were used to analyze quantitative and categorical variables respectively. Baseline characteristics and stimulation parameters were measured: age, BMI, duration of infertility, length of stimulation, antral follicle count(AFC), dose of rFSH and dFSH, pre-ovulatory follicle count(PFC), E_2 serum level previous to trigger, follicular output rate(-FORT), oocytes retrieved, metaphaseII oocytes, cleaved embryos, and endometrium thickness.
Main results and the role of chance: A total of 50 women (100 cycles) were included. We present the following values as mean± standard deviation: Age 32,9 (SD±), BMI 24,7 (4,2), AFC 20,8 (6,2), duration of infertility 34,7 months (19,1). Hormones were measured at cycle day 2-3 before starting each stimulation: AMH 6,4 (3,8). The mean individualized starting dose of rFSH was 149,3IU (45,6) whereas for dFSH was 7,7IU (1,9).
We observed significant difference between the length of the stimulation being shorter the rFSH one (**9,4**Vs.10,2 ***p0,031***) but no difference was found when comparing the use of dual trigger: Triptorelin plus Human chorionic gonadotropin (11 Vs.15 *p0,360*). We found no significant difference according to antagonist administration days (5,4Vs.5,04 days *p0,134)*, endometrial thickness (9,2Vs9,6 *p0,603*), PFC (10,1Vs.9,54 *p0,512*), FORT (53,7%Vs.48,7% *p0,616*), punctured follicles (15,1Vs.13,7 *p0,153*), retrieved oocytes (11,4Vs.10,2 *p0,246*), metaphase II oocytes (8,1Vs.7,1 *p0,193*), total cleaved embryos (1,9Vs.1,8 *p0,193*). No significant difference was observed between groups regarding cumulative pregnancy rate (34,4% Vs. 13,1%, *p0,08*).
However, we observed significant differences when comparing the E_2 serum levels (2600ng/mlVs.**1867ng/ml, *p0,031***) significantly lower with dFSH, number of grade A fresh embryos (0,06 Vs**. 0,2 *p0,045***), greater rate of fresh embryo transfer (38,8%Vs.**60,4% *p0,030***) and lower embryo freezing rate (30,6%Vs. **14,6% *p0,041***).
Limitations, reasons for caution: In these types of studies the investigator may incur in some manipulation about the exposure, as each subject acts as their own control. The degree of certainty also decreases since between pre-test and post-test there could have been other circumstances that alter the results. Also the population size is small.
Wider implications of the findings: dFSH might be useful in selected patients at risk of high response to stimulation in which we seek to minimize risks but still guaranteeing good results. Lower E_2 levels, greater rate of fresh embryo transfer and lower freezing rate were obtained in high responders
Trial registration number: 123

P-634 Transrectal ultrasound-guided oocyte retrieval and a successful IVF treatment outcome in a patient with vaginal agenesis.

D. Makhadiyeva[1], A. Ibragimov[1]

[1]Ecomed clinic, Reproductive medicine, Nur-Sultan, Kazakhstan

Study question: To report a case of a transrectal approach for an oocyte retrieval procedure in a patient with vaginal agenesis receiving *in-vitro* fertilization (IVF) treatment.
Summary answer: Transrectal ultrasound(US)-guided oocyte retrieval could be a valuable approach to achieve IVF pregnancy with a surrogate mother in a patient with complete vaginal agenesis.
What is known already: Despite of inability to child bear in the majority of patients with vaginal agenesis due to absence of normal reproductive tract, medically assisted reproduction is possible. Ovaries are usually present and patient's oocytes could be harvested and used in IVF. The challenge lies in oocyte retrieval procedure with complete absence of vagina. Abdominal US-guided oocyte retrieval including percutaneous transvesical, transurethral transvesical and transabdominal-transperitoneal have been previously reported as well as laparoscopy-guided oocyte retrieval in a patient with Müllerian agenesis. As of 2019 there have not been any published reports of transrectal approach in oocyte retrieval in humans.
Study design, size, duration: Case report of a patient with vaginal agenesis who undergone IVF treatment for primary infertility at a private reproductive medicine clinic.
Participants/materials, setting, methods: A 35-year old patient with a history of vaginal agenesis and hysterectomy presented with primary infertility. She was considered for transrectal US-guided oocyte retrieval and IVF with a standard controlled ovarian stimulation with recombinant follicle stimulating hormone and luteinizing hormone covered by gonadotropin-releasing hormone (GnRH) antagonist. When follicles were >15mm in mean diameter recombinant human chorionic gonadotropin (hCG) was administered to retrieve oocytes 36 hours later. A 22-year old woman served as a surrogate mother.
Main results and the role of chance: Following two short protocols of an ovarian stimulation and special preoperative bowel preparation with cleansing enema 5 oocytes (3 in the first cycle and 2 in the second cycle) were recovered transrectally under general anaestesia and subjected to IVF. There were no immediate or delayed postoperative complications. Transrectal approach for oocyte recovery was performed by an experienced urology and reproductive medicine specialist who had been operating transrectally urological patients in the past.
Normal fertilization was confirmed in 4 oocytes the next day. There were four (two in each IVF cycle) 6-9 cell Grade I embryos vitrified on day three postoocyte retrieval procedure. A few months later frozen-thawed embryo transfer of two embryos was performed following endometrial preparation with oestrogen in a healthy surrogate mother. A singleton intrauterine clinical pregnancy was confirmed four weeks later. Hormonal supplementation was continued until antenatal booking appointment. The pregnancy was uncomplicated and a healthy baby boy was delivered at term in August 2019.
Limitations, reasons for caution: a unique case of a rare condition
Wider implications of the findings: Transrectal approach may be useful in a select number of patients with vaginal agenesis who could not be eligible for previously reported abdominal methods of oocyte retrieval.
Trial registration number: not applicable

P-635 Premature ovarian insufficiency patients with viable embryos derived from autologous oocytes through repeated oocyte retrievals could obtain reasonable cumulative pregnancy outcomes following frozen-embryo transfer

X. Zhu[1], Y. Fu[2]

[1]Shanghai Ninth People's Hospital, Department of Assisted Reproduction, Shanghai, China ;
[2]Shanghai First Maternity and Infant Hospital, Department of Assisted Reproduction, Shanghai, China

Study question: To estimate cumulative pregnancy outcomes following frozen-embryo transfer (FET) in premature ovarian insufficiency (POI) patients undergoing in-vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) treatments with autologous oocytes.
Summary answer: Cumulative pregnancy outcomes following FET were reasonable for POI patients using autologous oocytes who could obtain viable embryos through repeated oocyte retrievals.
What is known already: Women with POI are often discouraged from using autologous oocytes because of the high cancellation rate during ovarian stimulation, the small number of available oocytes, the potential for poor oocyte quality, and the large economic burden in IVF cycles. Thus, oocyte donation is recommended to increase the chances of pregnancy in this population, which is still limited or even prohibited because of cultural, religious, or ethical issues in some countries. Additionally, some POI patients have a strong desire to be genetically linked to their offspring and insist upon pursuing IVF with their own eggs, despite the slim chances of success.
Study design, size, duration: This was a retrospective case-control study conducted in Department of Assisted Reproduction of the Ninth People's Hospital affiliated with Shanghai JiaoTong University School of Medicine between January 2012 and June 2019. Only patients undergoing IVF/ICSI treatments with a freeze-all strategy were screened, and 103 POI patients were matched with 515 normal controls in terms of the same number of viable embryos at the same embryonic age.
Participants/materials, setting, methods: The number of viable embryos was defined as the sum of all embryos obtained through repeated oocyte retrievals in POI patients, while it referred to embryos obtained from the first oocyte retrieval cycle at our centre for the control group. The embryonic age in the control group was considered as the age of women when the first viable embryo was obtained. The main outcome was cumulative clinical pregnancy rate (CCPR) following FET per patient.
Main results and the role of chance: Patients with POI and normal ovarian reserve had a comparable CCPR of 62.14% (64/103) and 65.24% (336/515), respectively (P = 0.547), and no statistical difference was found in the cumulative live-birth rate (CLBR) between the 2 groups (43.69% vs. 53.01%, P=0.084). Based on binary logistic regression, the CCPR and CLBR showed no association with the group (POI or normal ovarian reserve), whereas the numbers of embryos per transfer and the sum of all viable embryos per patient were positively associated with the CCPR and CLBR. The increase in the age of viable embryos was negatively related to the CLBR (OR, 0.944; 95% CI, 0.904–0.986; P=0.01 for the unadjusted model, and OR, 0.944; 95% CI, 0.903–0.986; P=0.009 for the adjusted model), while it had no relation to the CCPR. The clinical pregnancy rate (CPR) per FET cycle was 38.17% for the study group and 52.1% for the control group, while the CPRs per OPU cycle in the 2 groups were 11.25% and 69.9%, respectively; both were statistically different (P < 0.05). Moreover, POI patients had a lower implantation rate (27.8% vs. 37.94%) and higher early miscarriage rate per transfer (26.76% vs. 15%) than patients in the control group (P<0.05).
Limitations, reasons for caution: Only POI patients from whom viable embryos could be obtained were included, and the sample size was small. In addition, the primary outcome was calculated by the number of patients in our study instead of the number of oocyte retrieval and FET cycles in other published articles.
Wider implications of the findings: Our data may provide valuable information for POI patients who insist on using their own oocytes, and the method of repeated oocyte retrievals and FET may be beneficial for the treatment in POI patients.
Trial registration number: Not applicable

P-636 Is successful oocyte retrieval dependent on physician experience in natural IVF cycles?

S. Ertas[1], M. Ateskan[2], O. Bulduk[3], I. Keles[4], B. Balaban[3], K. Yakin[4]

[1]American Hospital, Women's Health Center, Istanbul, Turkey ;
[2]American Hospital, Asisted Reproduction Unit, Istanbul, Turkey ;
[3]American Hospital, Assisted Reproduction Unit, Istanbul, Turkey ;
[4]Koc University, Assisted Reproduction Unit, Istanbul, Turkey

Study question: Should follicle aspirations in natural IVF cycles be performed by only experienced clinicians?
Summary answer: Junior physicians are as good as their seniors are and even more tenacious in retrieving oocytes that develop into transferrable embryos in natural IVF cycles.
What is known already: Inexperienced clinicians may fail to retrieve the one and only oocyte available during natural cycle ICVF treatment.
Study design, size, duration: A retrospective analysis of 226 oocyte pick-up (OPU) procedures performed for natural IVF cycles between 2016-2018. Data regarding female age, time spent for oocyte retrieval, physician performing the procedure, oocyte maturity, fertilization, embryo transfer and pregnancy were extracted from the electronic database.
Participants/materials, setting, methods: Physicians were categorized based on their years of experience as senior (≥10 years, n=6), intermediate (1-10 years, n=4) and junior (<1 year, n=6). The time spent for oocyte retrieval was calculated from the entrance and withdrawal of the aspiration needle through the vaginal fornix. All OPU procedures were performed using a double lumen 17G needle and 180 mmHg aspiration pressure, under general anesthesia. Student's t test and chi-square test were used where appropriate.
Main results and the role of chance: Out of 226 OPU procedures, a single oocyte was harvested in 179 (79.2%) and the oocyte was in M-II stage in 156 (69%). The mean female age was 40.1 ± 4.6 years (27-46). The mean duration of the procedure was 586.6 ± 270.7 seconds (126-1288) and it was shorter (537.2 ±249.1 versus 777.7 ± 269.1 seconds, p<.001) when the oocyte could be retrieved. The rate of successful retrieval and the mean time to retrieve the oocyte were similar among physicians irrespective of their experience however, senior physicians terminated the procedure earlier when no oocyte could be obtained. The fertilization rate was 52.6% (82/156). Neither the rate of fertilized oocytes nor transferrable embryos were related with the experience of physicians. Among 66 women who had embryo transfer, 10 (15.1%) were pregnant.
Limitations, reasons for caution: This is a retrospective analysis from a single center and does not intend to confirm any causality.
Wider implications of the findings: Junior physicians are on a par with their seniors in retrieving oocytes in natural IVF cycles.
Trial registration number: Not applicable

P-637 Herbal medicine in women undergoing in vitro fertilization/ intracytoplasmic sperm injection: A systematic review and meta-analysis

J.Y. Jo[1], C.Y. Kwon[2], B. Lee[3], S.H. Lee[4]

[1]Conmaul Hospital of Korean Medicine, Korean Obstetrics and Gynecology, Seoul, Korea- South ;
[2]Kyung Hee University, Clinical Korean Medicine- Graduate School, Seoul, Korea- South ;
[3]Korea Institute of Oriental Medicine, Clinical Medicine Division, Daejeon, Korea- South ;
[4]Kyung Hee University, Pediatrics of Korean Medicine, Seoul, Korea- South

Study question: Does herbal medicine (HM) improve the live birth rate (LBR) and clinical pregnancy rate (CPR) in infertile women undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI)?
Summary answer: Our analysis finds a benefit of HM in women undergoing IVF/ICSI.
What is known already: Combination of IVF and HM has widely used in Asian countries. A previous systematic review suggested that combination of IVF and CHM used in the included trials improve IVF success, however, high risk of bias observed with the trials and there were no studies that reported live birth rate (LBR).
Study design, size, duration: A systematic review and meta-analysis of randomized controlled trials (RCTs) (until September 2019) that evaluated the effects of HM as an adjunct to IVF was conducted.

Participants/materials, setting, methods: Studies were considered for inclusion if they were RCTs; included infertile women who were undergoing IVF/ICSI; compared the effectiveness of HM administrated immediately before, during and/or after IVF with placebo, no treatment, or other active treatments; and reported at least one of the pregnancy outcomes including LBR, ongoing pregnancy rate, and CPR.
Main results and the role of chance: From a total of 43 RCTs involving 4316 participants, HM was more likely to increase LBR (5 studies; risk ratio (RR) 1.34, 95% confidence intervals (CI): 1.05 to 1.72, P = 0.02) and CPR (35 studies; RR 1.38, 95% CI: 1.29 to 1.49, P < 0.00001) than not receiving adjunctive treatment. The CPR in HM treatment group was also improved when compared with that of the placebo group (5 studies; RR 1.85, 95% CI: 1.42 to 2.42, P < 0.00001). However, compared with active controls, HM did not significantly improved CPR (RR 1.19, 95% CI: 0.71 to 2.01, P = 0.51). Sensitivity analyses restricting to RCTs with high quality did not influence the results: HM improved LBR (2 studies; RR 1.86, 95% CI 1.24 to 2.80, I^2=0%) and CPR (30 studies; RR 1.42, 95% CI 1.30 to 1.54, I^2=0%), compared to no adjunctive treatment. HM also increased CPR (2 studies; RR 1.77, 95% CI 1.19 to 2.63, I^2=0%), compared to placebo. Reported adverse events such as miscarriage, ectopic pregnancy rate and incidence of ovarian hyperstimulation syndrome were not significantly different between the HM and control group.
Limitations, reasons for caution: Given poor reporting and methodological weaknesses of the existing studies, large-scale, long-term RCTs with rigorous methodological input are needed to clarify the role of HM in this population.
Wider implications of the findings: There is promising evidence from the currently available RCTs to judge the effectiveness and safety of HM on pregnancy or childbirth outcomes in women undergoing IVF.
Trial registration number: not applicable

P-638 Late-follicular phase elevated serum progesterone is associated with embryo quality but not cumulative live birth in IVF/ICSI

C. Minghui[1], G. Jun[1], L. Lu[1], M. Qingyun[1], X. Yanwen[1], C. Zhou[1]

[1]the First Affiliated Hospital of Sun Yat-sen University, Reproductive Medicine Centre, Guangzhou, China

Study question: Is late-follicular phase elevated serum progesterone (LFEP) associated with cumulative live birth when freeze-all strategy was adopted in patients with LFEP during IVF/ICSI?
Summary answer: Late-follicular phase elevated serum progesterone is not associated with cumulative live birth when freeze-all strategy was adopted in patients with LFEP during IVF/ICSI.
What is known already: Late-follicular phase elevated serum progesterone during IVF/ICSI detriment endometrium receptivity and impede embryo implantation. It is unclear whether late-follicular phase elevated serum progesterone still disadvantage cumulative live birth when freeze-all strategy was adopted in patients with late-follicular phase elevated serum progesterone during IVF/ICSI.
Study design, size, duration: This is a retrospective cohort study; A total of 6076 ovarian stimulation cycles and 5461 women who underwent IVF/ICSI treatment in our hospital from January 2016 to December 2017 were identified and reviewed.
Participants/materials, setting, methods: All the embryos were freezed in cycles with progesterone levels on trigger day higher than 1.50 ng/ml. Cumulative live birth rate was defined as the first live birth per ovarian stimulation cycle including fresh and frozen cycles and evaluated by group. Multivariable linear regression and binary logistic regression was used to assess the association between progesterone levels on trigger day and embryo quality and cumulative live birth after adjusting for confounding factors.
Main results and the role of chance: The cumulative live birth rate in cycles using GnRH agonist protocol was 52.7%, 58.3%, 59.9%, 49.2% and 50.0% (P=0.008); the CLBR in cycles using GnRH antagonist protocol was 38.5%, 51.2%, 51.7%, 62.9% and 37.5% (P<0.001) with progesterone level on trigger day ≤0.50, 0.51–1.0, 1.01-1.50, 1.51-2.00 and ≥2.01 ng/ml respectively. Progesterone level on trigger day was not associated with cumulative live birth (OR 0.946, 95%CI 0.773-1.158, P=0.589) after adjusting female age, thickness of endometrium on the hCG trigger day, ovarian stimulation protocol, the number of viable embryo and high-quality embryo. The progesterone level on trigger day was negatively associated with the number of viable embryo (B=-0.513, P<0.001) and high-quality embryo (B=-0.492, P<0.001) after adjusting female age and the number of mature oocyte.
Limitations, reasons for caution: As a retrospective study, our analysis depended on previously recorded data. The conclusion is limited to achieve one live birth per ovarian stimulation cycle.
Wider implications of the findings: Although late-follicular phase elevated serum progesterone does not affect the possibility to achieve one live birth per ovarian stimulation cycle, it may adversely affect the total live birth per ovarian stimulation cycle because it was associated with a decrease in number of viable embryo and high-quality embryo.
Trial registration number: N/A

P-639 Segmentation (elective freeze all) results in higher clinical pregnancy rates in women with high AMH (> 30 pmol/L)

F. Roebuck[1], P. Ambrose[2], L. Munn[2], T. Hamilton[2], C. Noble[1], J. Lyon[3], M. Gaudoin[4], R. Fleming[3]

[1]GCRM Fertility, Embryology, Glasgow, United Kingdom ;
[2]GCRM Fertility, Nursing, Glasgow, United Kingdom ;
[3]GCRM Fertility, Biochemistry, Glasgow, United Kingdom ;
[4]GCRM Fertility, Medical, Glasgow, United Kingdom

Study question: To determine if segmentation in high responder women resulted in higher clinical pregnancy rates (CPR) in the first frozen embryo transfer than with fresh transfer.
Summary answer: The CPR was higher in the first frozen embryo transfer (FET) compared to the fresh transfer in women with high AMH levels (P < 0.001).
What is known already: Pregnancy rates appear to be lower in GnRH-antagonist-controlled cycles, possibly because of greater endometrial sensitivity to circulating progesterone (Bosch et al, Fertil Steril, 2015).
Segmentation is probably not beneficial in ovulatory women with normal AMH levels (Vuong et al, NEJM, 2018) but might be beneficial in anovulatory women, especially as this latter group has an increased risk of ovarian hyperstimulation syndrome with a fresh embryo transfer (Roque et al, Hum Reprod. Update, 2019).
Study design, size, duration: Historical data from our centre showed a very modest CPR with fresh embryo transfer in women with high AMH using GnRH-antagonist control but substantially higher CPR with their subsequent frozen transfer.
From January 2019 we prospectively elected to culture all such patients to blastocyst and vitrified all suitable embryos for a subsequent frozen embryo transfer to determine if the subsequent first frozen transfer was superior to the historical fresh transfer outcomes.
Participants/materials, setting, methods: All patients with AMH ≥ 30 pmol/L received Menopur (Ferring Pharmaceuticals) using GnRH-antagonist control. The primary outcome was CPR in the women having a GnRH-trigger, undergoing segmentation and having their first frozen embryo transfer from January – October, 2019 (N = 81, Group-Seg^n). This was compared with the historical outcomes of the same subgroup of women in the preceding 2 years (but with HCG triggering) and a fresh embryo transfer (N = 158, Group-Hist).
Main results and the role of chance: There was no difference in age (Group-Hist = 34.1 ± 4.5 years, Group-Seg^n = 34.6 ± 3.7 years, P = 0.39), BMI (25.7 ± 7.0 vs. 26.5 ± 6.4, P = 0.39) or AMH (48.5 ± 21.0 vs. 46.5 ± 18.8, + P = 0.47). Group-Seg^n stimulated for longer (9.4 ± 2.2 days vs. 8.5 ± 0.7, P < 0.001), received more FSH (1402 ± 324 vs. 1275 ± 106, P < 0.001) and more eggs were retrieved (16.6 ± 8.4 vs. 13.6 ± 6.8, P = 0.003). Group-Seg^n got more 2PN embryos (10.1 ± 6.0 vs. 8.5 ± 5.3, P = 0.035) but there was no difference in the mean number of embryos transferred (1.1 ± 0.3 vs. 1.2 ± 0.8, P = 0.28). Group-Seg^n had a higher implantation rate (IR, 48% vs. 26%, P < 0.001) and higher CPR (heartbeat on scan at 8 weeks' gestation, 52% vs. 27%, P < 0.001).
This was a prospective study comparing an additional intervention with historical data. The groups appeared to be evenly matched morphometrically although, because there was little fear of ovarian hyperstimulation syndrome when using a GnRH-agonist trigger, Group-Seg^n were stimulated for longer and received more FSH.
Limitations, reasons for caution: We cannot as yet determine if the cumulative pregnancy rate/live birth rate from a single fresh treatment cycle will be equivalent.

Wider implications of the findings: In our study population, segmentation conferred benefit in terms of IR and ongoing CPR compared with a fresh embryo transfer. Our study population was older than other widely-cited studies so it is imperative that individual clinics examine their internal data to determine if a segmentation approach might benefit their patients.
Trial registration number: N/A

P-640 Ovarian sensitivity index -a novel marker of ovarian responsiveness in IVF cycles

C. JANDIAL[1], S. Malik[1], V. Bhatia[1], V. Prakash[1], S. Talwar[1]
[1]southend Fertility and IVF - NEW DELHI, IVF, NEW DELHI, India

Study question: To validate the use of OSI as measure of ovarian response during IVF cycles by correlating it with age, AMH ,AFC , total dose of gonadotropins and quality of embryos
Summary answer: OSI can be used as dynamic marker for ovarian reserve, a guide for gonadotropin dose in future IVF cycles and predictor of good quality embryos
What is known already: In Controlled Ovarian Stimulation, different women respond differently to similar doses of gonadotropins . Preliminary studies have suggested that a threshold level of gonadotropins may exist and no more competent oocytes are obtained if exceeded. Recent studies, on the contrary, indicate that high ovarian response to gonadotropins is not so detrimental. The key point is , not the gonadotropin dose or ovarian response alone, but a combination of these two , is important .This is the concept addressed in the ovarian sensitivity index. High OSI means, more oocytes are retrieved with fewer gonadotropin doses and offers best pregnancy outcomes
Study design, size, duration: This is a retrospective, single centre study which included 256 women over a period of one year.
Participants/materials, setting, methods: Retrospective analysis which included 256 women with age < 42 years , no previous history of ovarian surgery, chemoradiation and had no endocrine disorders. Baseline scan was done to determine the AFC. Subjects were treated with either long agonist / antagonist protocol as per clinical and biochemical markers. OPU was done 36 hours after trigger injection. Ovarian sensitivity Index was calculated and corelated with age, AMH, AFC, Total dose of Gonadotropins and top quality embryos
Main results and the role of chance: Out of 256 women enrolled in the study with a mean age of 32 years,175(68.36%) were a case of primary subfertility with an average duration of 4 years.208 women received antagonist protocol .Mean number of oocytes retrieved were 8.Statistical analysis as per Pearson's Correlation Coefficient showed a negative correlation of OSI with age and positive correlation with AMH and AFC (p value-0.0000).Positive correlation (0.8162,p value 0.000) was observed with number of oocytes recovered and top quality embryos (0.4 and 0.3,p values 0.0000 and 0.0000 respectively. Further analysis of data revealed that as OSI increases the mean number of top quality embryos increases
Limitations, reasons for caution: A retrospective analysis, single centre, smaller group and includes patients with different protocols
Wider implications of the findings: In failed IVF cycles, management of future cycle can be guided by OSI. Patients with higher values should receive same dose of FSH in future cycles while those with low OSI should receive higher dose of FSH. OSI can be used as a predictor of good quality embryos.
Trial registration number: NOT APPLICABLE

P-641 Prospective multi-centre non-interventional study to evaluate the application of individualized follitropin delta treatment in routine clinical practice.

C. Blockeel[1], P. Wijngaard-Boom[2], G. Griesinger[3], P. Larsson[4], Y.L. Yip Sonderegger[5], F. Beligotti[6], S. Riviere[6]
[1]Universitair Ziekenhuis Brussel, Centre for Reproductive Medicine, Jette- Brussels, Belgium ;
[2]Erasmus Medical Center, Division of Reproductive Medicine- Department of Obstetrics and Gynecology, Rotterdam, The Netherlands ;
[3]University Hospital of Schleswig-Holstein, Department of Gynecological Endocrinology and Reproductive Medicine, Luebeck, Germany ;
[4]Ferring Pharmaceuticals, Biometrics - Global Clinical R&D, Copenhagen, Denmark ;
[5]Ferring Pharmaceuticals, Global Market Access & Pricing, Saint-Prex, Switzerland ;
[6]Ferring Pharmaceuticals, Global Medical Affairs, Saint-Prex, Switzerland

Study question: To investigate the use of follitropin delta, a human cell line derived recombinant human Follicle Stimulating Hormone with individualised dosing regimen, in routine clinical practice.
Summary answer: The use of follitropin delta, in routine care setting with a broad and varied patient population, shows results aligned with the ESTHER-1 registration trial (NCT01956110).
What is known already: When compared to Chinese-hamster ovary (CHO) derived follitropin alfa, the individualised dosing of follitropin delta resulted in ESTHER-1 in: similar pregnancy rates; less frequent measures to prevent ovarian hyperstimulation syndrome (OHSS); more women with a target ovarian response (8–14 oocytes); fewer poor responses with anti-Müllerian Hormone AMH<15pmol/L; fewer excessive responses with AMH≥15pmol/L. For the follitropin delta arm, mean total dose was 90±25.3mcg, mean stimulation was 8.9±1.9days, mean number of oocytes was 10.0±5.6; 43.3% with 8-14 oocytes, 8.0±4.3 with AMH<15pmol/L (44.1%), 11.6±5.9 with AMH≥15pmol/L (55.9%). The clinical pregnancy rate was 34.9%, and the OHSS rate (all, any grade) was 3.5%.
Study design, size, duration: Interim analysis including 390 patients of a prospective multicentre non-interventional study using data collected from routine clinical practice performed across 38 sites in 10 countries. Women naïve to in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) were included in this study. Women who did not achieve pregnancy could continue with the same treatment for up to three cycles. The study is still recruiting; 1300 patients are anticipated over a period of 3 years (2018-2020).
Participants/materials, setting, methods: Women are prescribed follitropin delta with an individualised daily dose using an approved algorithm based on the patient's Body weight (BW) and serum AMH levels measured by Elecsys® AMH plus (Roche Diagnostics). The decision to treat patients with follitropin delta is independent from the decision to enrol the patient into the study. During the observation period, the investigators collected data from the routine practice.
Main results and the role of chance: This interim evaluation included 390 patients with mean (SD) age 33.3±4.7 years, BW 67.3±12.7 kg, and AMH 20.4±15.2 pmol/L. The total dose determined with the algorithm was 102.6±33.5 mcg, mean treatment duration was 9.9±2.3 days. Both Gonadotropin-releasing hormone (GnRH) antagonist (84.9%) or GnRH agonist (15.1%) protocols were used. Cycle cancellation occurred in 19 patients prior to oocyte retrieval, mainly due to poor ovarian response. Final oocyte maturation occurred in 377 patients: 82.2% with human chorionic gonadotropin (hCG), 13.8% with GnRH agonist and 4.0% with dual trigger. Per patient who received triggering of final follicular maturation, mean number of collected oocytes was 10.5±6.2: 9.7% with <4 oocytes, 25.3% with 4-7 oocytes, 40.4% with 8-14 oocytes, 15.9% with 15-19 oocytes and 8.6% with ≥20 oocytes. Mean number of retrieved oocytes was 7.7±4.1 with AMH <15 pmol/L (43.3%), 12.7±6.7 with AMH ≥15 pmol/L (56.6%). The majority of patients had embryo transfer with fresh embryos (84.4%) on day 5 (54.7%). Per started cycle, 32.3% had a positive hCG test and 27.6% had a clinical pregnancy. In total, 4.1% of OHSS cases (all, any grade) were reported.
Limitations, reasons for caution: This interim evaluation including 390 patients, who started and finished (with an ovum pick-up or with cycle cancellation) a controlled ovarian stimulation treatment for IVF or ICSI, provides results from the first treatment cycle. The whole data-set (1300 patients) should confirm these findings and allow further exploration of patients' outcomes.
Wider implications of the findings: Individualised follitropin delta treatment of a broad patient population, as presented in the real-world clinical setting, reflects a variety in the treatment approaches. It also confirms that this new dosing regimen based on AMH levels and BW remains predictable and allows targeting the desired ovarian response for naïve patients.
Trial registration number: NCT03393780

P-642 Clinical relevance of serum progesterone level on the day before frozen embryo transfer when using a combined luteal phase support.

N. Ramos Seixas[1], P. Pirtea[1], A. Benammar[1], D. De Ziegler[1], R. Frydman[1], M. Poulain[1], J.M. Ayoubi[1]
[1]Hôpital Foch, Service de Gynécologie et Obstetrique, Paris, France

Study question: To determine serum progesterone levels (SPL) with a combined administration (subcutaneous and vaginal progesterone) and its impact on pregnancy outcomes in frozen embryo transfer (FET).

Summary answer: We reported that the association of vaginal and subcutaneous progesterone for luteal phase support (LPS) allows achieving optimal progesterone levels before FET for most patients.

What is known already: ART outcomes following FET depends on progesterone support efficiency. As previously reported, low SPL on the day of embryo transfer is associated with lower outcomes in FET. Different SPL thresholds have been described as having an impact on pregnancy rates. While Labarta reported decreased implantation rate for SPL under 9.2ng/ml after vaginal administration, Yovich found a lower implantation rate once SPL was lower than 15.7ng/ml or above 31ng/ml with a pessary vaginal regimen. Alur-Gupta reported lower pregnancy rates with intramuscular progesterone LPS when SPL were under 15ng/ml. No study has described SPL after combined subcutaneous and vaginal progesterone administration.

Study design, size, duration: Retrospective study in a public academic ART center between February 2019 and November 2019 including all single frozen-thawed blastocyst transfers for women up to 43 years of age. We included 231 FET cycles for 198 patients for data analysis.

Participants/materials, setting, methods: Patients included had a normal uterine cavity. They received hormonal replacement therapy (HRT) with a step-up estradiol (E2) oral doses protocol, starting with 4 mg/day, reaching 8mg/day on day 9.

After achieving appropriate endometrium thickness (>7mm), daily subcutaneous progesterone 25 mg and 800mg vaginal progesterone were introduced. FET was performed after 5 days of progesterone administration. SPL was controlled one- or two-days prior FET. Statistical analysis was performed using the two-sample Student t-test.

Main results and the role of chance: We included 231 FET cycles for 198 patients for data analysis. Main results showed a mean SPL of 34.8ng/ml, on the day before FET. The lowest value of SPL was 7.4ng/ml and the highest was 145.7ng/ml. With this combined protocol for LPS, only 1% of patients had progesterone levels under 9.2ng/ml, when they were 25% in Labarta's study with vaginal protocol support. This indicates that the combination of subcutaneous and vaginal progesterone as luteal phase support is efficient for this population.

We obtained a clinical pregnancy rate (CPR), defined as the presence of an embryo with cardiac activity of 29%. The miscarriage rate (clinical pregnancy loss before 12 weeks) was 18%. The ongoing pregnancy rate (OPR), (CPR after 12 weeks) was 24.7% (OPR/FET).

The mean SPL was significantly higher in the group with an OPR in comparison with those in the miscarriage group (41.1 ±30.9 vs. 23.3 ±14.5, p<0.01; CI 95%). And the OPR was significantly higher for patients presenting a SPL above 22.6ng/Ml compared to the ones underneath (19.0% vs. 30.4%, p<0.05; CI 95%), even though there was no statistical difference in terms of: age, E2 levels, endometrial thickness or embryo morphology between the two groups.

Limitations, reasons for caution: An important limitation of this study is that, as a retrospective and observational study, we couldn't compare the serum progesterone levels to a reference population having a different luteal phase support regimen.

Wider implications of the findings: These findings support the value of a combined progesterone regimen for LPS in FET, providing optimal levels of progesterone. Further studies with a larger population and a control group should be performed in order to confirm our hypothesis.

Trial registration number: Not applicable

P-643 Validation study of the stability of serum progesterone following cryo-conservation and the performance of standard immunoassay for quantifying supra-physiological serum progesterone levels following ovarian hyperstimulation

L. Thomsen[1], U. Kesmodel[2], P. Humaidan[1]

[1]Department of Clinical Medicine- Aarhus University- Denmark., The fertility Clinic-Skive Regional Hospital- Denmark., Skive, Denmark ;
[2]Department of Clinical Medicine- Aalborg University- Denmark., The fertility Unit- Aalborg University Hospital- Denmark., Aalborg, Denmark

Study question: Is standard immunoassays for progesterone (P4) measurements accurate in IVF-patients with supra-physiological P4-levels? Furthermore, are serum P4-levels stable over time when cryo-conservation is used?

Summary answer: The accuracy of a standard immunoassay is maintained in the P4-concentration range seen in IVF-patients. Furthermore, serum P4-levels are stable after 2.5 years of cryo-conservation.

What is known already: Several studies both in fresh IVF-cycles and frozen embryo transfer cycles have demonstrated the importance of luteal P4-levels on the chance of live birth. Serum P4-levels are easily obtained using standard immunoassays. However, consideration is required when a standard assay validated for measurements in a physiological P4-range in a normal population (i.e. during the natural cycle) is used in an "abnormal" population (IVF-patients) with supra-physiological P4-values. Furthermore, in large clinical trials study samples are cryo-conserved for several years before analysis. Current knowledge about the long-term stability of P4 in human serum is rather incomplete and, in part, contradictory.

Study design, size, duration: Validation study of laboratory factors with a possible impact on the interpretation of luteal P4-levels following ovarian stimulation: First, a test of the precision of P4-measurements in supra-physiological P4-ranges using a standard immunoassay (Siemens Immulite 2000XPi). Secondly, an assessment of the possible changes in serum P4-levels following cryo-conservation at -80°C during a 2.5-year period. Serum samples from the luteal phase were obtained from 602 IVF patients to assess the P4-range following ovarian hyperstimulation.

Participants/materials, setting, methods: Serum samples were pooled in three pools to reach a P4-concentration of roughly 125, 300 and 600 nmol/l, respectively. Following mixture, 10 serum samples from each pool were collected and frozen. During ten successive days, one sample from each pool was analyzed and the coefficients of variation (CV) were calculated. Another 60 luteal serum samples were analyzed at baseline, after 9 months and after 2.5 years to assess the long-term stability of P4-concentrations in serum.

Main results and the role of chance: The 602 serum samples for P4-measurement from the early or mid-luteal phase ranged from 19 nmol/l to 1224 nmol/l, thus up to x20 times the levels seen during the natural cycle. The inter-assay and intra-assay precision for the Siemens Immulite stated by the supplier based on measurements within the normal range of a menstrual cycle is <7%. The CV values for the sample pools (125 nmol/l, 300 nmol/l and 600 nmol/l) were 7%, 5% and 4%, respectively. In conclusion, the experimental runs of test samples showed that the precision of the used immunoassay is maintained in the P4-concentration ranges seen in IVF-patients following ovarian stimulation. Thus, luteal P4-levels in IVF-patients can be assessed by standard immunoassay quantification with sufficient accuracy and without the risk of misclassification bias.

To evaluate the effect of storage on the serum P4-levels, a test run of 60 serum samples was performed. The test runs showed comparable levels of P4 in study samples compared to the baseline results. The CV-value for P4-measurements performed at baseline and the first re-run (9 months) was 3.4±2.9% and 2,7 ±2.4% for the second re-run (2.5 years). Thus, the changes in P4-concentrations remained within the expected variation for the immunoassay.

Limitations, reasons for caution: The results are based only on measurements performed by Siemens Immulite 2000XPI. However, automated immunoassay analyzers are comparable in performance and precision, and therefore the results are expected to cover other immunoassay analyzers as well.

Wider implications of the findings: The precision of a standard immunoassay is maintained in supra-physiological P4-levels in IVF-patients and can be used in clinical and study set-ups without the risk of misclassification bias. Short-term cryo-conservation does not change serum P4-levels. Study samples can therefore be frozen and stored until analysis without compromising P4 test results.

Trial registration number: NCT02129998

P-644 Outcome of in vitro oocyte maturation (IVM) in patients with PCOS: does patient phenotype have an impact?

M. De Vos[1,2], S. Mackens[1], S. Pareyn[1], P. Drakopoulos[1], T. Deckers[3], L. Mostinckx[1], I. Segers[1], S. Santos-Ribeiro[4], M. Camus[1], G. Verheyen[1], H. Tournaye[1], C. Blockeel[1]

[1]Universitair Ziekenhuis Brussel, Centre for Reproductive Medicine, Brussel, Belgium ;
[2]Vrije Universiteit Brussel, Follicle Biology Laboratory FOBI, Brussels, Belgium ;
[3]AZ Sint-Lucas, Gynaecologie-Verloskunde, Brugge, Belgium ;
[4]IVI-RMA Lisboa, Gynecology/Reproductive Medicine, Lisbon, Portugal

Study question: Does the phenotype of patients with polycystic ovary syndrome (PCOS) affect clinical outcomes of assisted reproductive technology (ART) following *in vitro* oocyte maturation (IVM)?
Summary answer: Cumulative live birth rates (CLBR) after IVM are significantly higher in patients with a hyperandrogenic PCOS phenotype compared to those with a normo-androgenic phenotype.
What is known already: Hyperandrogenic PCOS phenotype has been reported to confer a lower CLBR after controlled ovarian stimulation (COS) and IVF/ICSI when compared to patients with a PCO-like ovarian morphology or with a normo-androgenic phenotype. Whether there is an influence of the different PCOS phenotypes on success rates of IVM remains unclear.
Study design, size, duration: This is a single-centre, retrospective cohort study including 320 unique PCOS patients performing their first IVM cycle between April 2014 and January 2018 in a tertiary referral hospital.
Participants/materials, setting, methods: Baseline patient characteristics and IVM treatment cycle data were collected. PCOS was diagnosed according to the Rotterdam criteria. The primary outcome was cumulative live birth rate (CLBR), defined as the rate of deliveries resulting from the transfer of all fresh and cryopreserved embryos from the same IVM cycle. The secondary outcome was live birth rate following the first IVM embryo transfer.
Main results and the role of chance: Half of the patients (n=160) presented with a hyperandrogenic PCOS phenotype. These patients had a higher BMI (27.4±5.4 kg/m^2 vs 23.3±4.4 kg/m^2, p<0.001) and used more often metformin (31.2% vs 11.2%, p<0.001) in comparison with the normo-androgenic ones. Significantly more cumulus oocyte complexes were retrieved in the hyperandrogenic group (24.9±18.3 vs 19.8±13.5, p= 0.003) and, after IVM, these patients had more mature oocytes (11.7±9.0 vs 9.1±6.9, p=0.002). Following the first embryo transfer, the positive hCG rate and LBR were comparable in the two patient groups (44.2% vs 36.7%, p=0.21 and 23.2% vs 26.6%, p=0.52, respectively). In hyperandrogenic patients, early pregnancy loss was more common (20.3% vs 10.2%, p= 0.02). The CLBR (when at least one embryo was available for transfer) was not significantly different following univariate analysis (42.7% vs 41.4%, p= 0.82). However, when using a multivariable logistic regression model to account for confounding factors, the PCOS phenotype appeared to be significantly correlated with CLBR, with more favorable results in the hyperandrogenic group (OR 2.24, CI 1.06-4.77, p= 0.03).
Limitations, reasons for caution: These data should be interpreted with caution in the light of the retrospective nature of the study and the possibility of unmeasured confounding.
Wider implications of the findings: Infertile hyperandrogenic PCOS patients undergoing IVM achieve a higher CLBR than their normo-androgenic counterparts. This is in contrast with previously reported outcomes following COS and ART in which hyperandrogenic PCOS patients performed significantly worse. Our data suggest that proper patient selection is of utmost importance in an IVM program.
Trial registration number: Not applicable

P-645 Anogenital distance in newborn infants conceived by assisted reproduction and natural conception

S. Ercin[1], S. Ertas[2], O. Dundar[3], O. Oktem[4], K. Yakin[2]

[1]American Hospital, Neonatology, istanbul, Turkey ;
[2]American Hospital, Women's Heath Center, Istanbul, Turkey ;
[3]American Hospital, Women's Health Center, Istanbul, Turkey ;
[4]Koc University, Women's Heath Center, Istanbul, Turkey

Study question: Does anogenital distance (AGD) differ in newborn infants conceived through assisted reproduction technologies (ART) compared with those conceived naturally
Summary answer: AGD measurements in infants conceived by ART are not different from those of infants conceived naturally
What is known already: AGD is an anthropometric measurement sensitive to changes in the in-utero hormonal environment during the early life of fetus. Alterations in AGD are associated with anomalies in neonates and reproductive dysfunction in adults
Study design, size, duration: A prospective descriptive cohort study was performed on randomly selected singleton neonates (n = 447) born at a single center between September 2017 and April 2019. Anthropometric and anogenital measurements were performed in 247 male and 200 female newborns born after ART (n=121) or natural conception (n=326), within 24 hours of birth by a single caregiver who was blind to the mode of conception.
Participants/materials, setting, methods: Gestational age, birth weight, length and head circumference were recorded for all newborns. In female infants, distance from anus to the anterior clitoris (AGDAC) and to the posterior fourchette (AGDAF) were recorded. In male infants, distance from the center of the anus to the posterior base of the scrotum (AGDAS) and to anterior base of the penis (AGDAP) were measured.
Main results and the role of chance: The mean age of mothers was 34.9 years and the mean gestational age at the time of delivery was 267 days. ART cohort was older, more likely to be nulliparous and delivered by cesarean section at an earlier gestational week. AGDAS of male infants was approximately twice as long as AGDAF of female infants (17.6±5.0 vs 9.1±3.6 mm, p<0.001). On average, female infants conceived by ART had shorter AGDAF (8.8±3.6 mm) than those conceived naturally (9.1±3.6 mm) but the difference was not significant (p >0.05). AGDAC were comparable for both groups (27.7 ± 7.1 vs 27.4 ± 6.3 mm, p>0.05). In male infants, no significant difference was seen between ART and natural conception groups in terms of AGDAS (17.4±4.6 vs 17.7±5.2 mm) and AGDAP (38.0 ± 6.7 vs 37.5 ± 6.6 mm, p >0.05 for both). Gestational age, weight, length and head circumference at birth were the strongest correlates of anogenital measurements. When adjusted for these covariates, mode of conception was not associated with differences in any of the anogenital measurements.
Limitations, reasons for caution: A single center study with relatively small sample size, it is not possible to rule out confounding by diet or endocrine-disrupting chemicals or drugs other than progesterone that women might have taken during pregnancy
Wider implications of the findings: Preliminary findings are reassuring for women undergoing ART but they need to be confirmed by future studies with larger sample sizes
Trial registration number: not applicable

P-646 Does quantity equal quality? – a morphokinetic assessment of embryos obtained from young women with decrease ovarian response to stimulation.

N. Schachter - Safrai[1], G. Karavani[1], Y. Kantor[2], I. Har- Vardi[3], A. Buxboim[2], Z. Shoham[4], Y. Or[4], A. Ben-Meir[1]

[1]Hadassah Hebrew University Hospital, Obstetrics and Gynecology, Jerusalem, Israel ;
[2]Institute of Life Sciences- The Hebrew University of Jerusalem, Alexander Grass Center for Bioengineering- School of Computer Science and Engineering, Jerusalem, Israel ;
[3]Soroka University Medical Center, The IVF Unit - Department of Obstetrics and Gynecology, Beer-Sheva, Israel ;
[4]Kaplan Hospital, Department of Obstetrics and Gynecology, Rehovot, Israel

Study question: Is there an association between oocyte quantity and quality in young women exhibiting decreased ovarian response?
Summary answer: The quantitative decrease in ovarian response is associated with reduced oocyte quality, reflected by a slower developmental rate and lower implantation and pregnancy rates.
What is known already: It is well known there is a physiologic decline in both oocyte quantity and quality attributed to aging. However, it is unclear if the two are related when a premature decline in oocyte quantity is observed. While most of the literature on this subject includes a heterogenous population and demonstrates conflicting results regarding embryo quality and pregnancy rate, a recent study included only women younger than 38 years with low ovarian response and demonstrated no difference in embryonic development and live birth rate .There is only scarce data regarding oocyte quality in poor responders using a time lapse microscopy (TLM).
Study design, size, duration: The study included a retrospective assessment of morphokinetic parameters performed by TLM from five medical centers between January 2013 and December 2017.The developmental process and kinetics of 519 embryos obtained from the study group, referred as the " decreased ovarian response" (DOR) group was compared to 3633 embryos of the control group ,known as the "normal ovarian response" (NOR) group.
Participants/materials, setting, methods: The study included patients younger than 38 years who underwent controlled ovarian stimulation (COS)

with consecutive aspiration of either 5 oocytes or less - the DOR group, or 6 oocytes or more- the NOR group. A comparison between the morphokinetic parameters, implantation and pregnancy rates of the two groups was made with additional subgroup analysis according to the implantation status. Logistic regression was conducted to assess the association between morphokinetic parameters and implantation rate

Main results and the role of chance: Implantation and clinical pregnancy rates were significantly lower in the DOR group compared to the NOR group (31.6% vs. 44.5% and 37.7% vs. 51.5%, respectively; p<0.05). Embryos from the DOR group reached the morphokinetic milestones later than embryos obtained from NOR patients. Implanted embryos in the DOR group developed faster than embryos which failed to implant, however manifested a protracted course compared with implanted embryos from the NOR group. In a multivariate analysis - normal ovarian response, age and tSB were associated with implantation.

Limitations, reasons for caution: The two patients' groups were not homogenous in their basic characteristics. A higher rate of blastocyst culture and blastocysts transfer was demonstrated in the NOR patients. Important information regarding the maximal dose of gonadotropins obtained, previous IVF response and ovarian reserve testing was lacking.

Wider implications of the findings: Decreased oocyte quality manifested by a multi-staged delayed preimplantation development in the DOR group may highlight another pathophysiology for decreased oocyte quality not related to age. Decreased ovarian response to COS and morphokinetic parameters may assist in predicting pregnancy.

Trial registration number: not applicable

P-647 Follicular fluid anti-mullerian hormone: a predictive marker of fertilization, implantation and clinical pregnancy in patients with unexplained infertility

A. Abdelmeged[1], O. Ahmed[1], A. Kotb[1], E. Elgindy[1], H. Baha[1]

[1]Faculty of Medicine, Gynacology and Obstetrics, El Menia, Egypt

Study question: Would follicular fluid anti-mullerian hormone (FF-AMH) measured values predict fertilization, implantation and clinical pregnancy in patients with unexplained infertility undergoing ICSI?

Summary answer: FF-AMH level could be associated with fertilization rate (FR), implantation rate (IR), and clinical pregnancy rate (CPR) in patients with unexplained infertility undergoing ICSI.

What is known already: In humans, AMH is considered a reliable marker for ovarian reserve, as well as for ovarian response to gonadotropin stimulation. AMH probably has a role in the regulation of follicle selection and maturation. FF-AMH inhibits the initiation of human primordial follicle growth and prevents multiple selections of a dominant follicle; by reducing their sensitivity to follicle stimulating hormone (FSH). Previous studies suggested that FF-AMH might be a better predictor of ovarian responses to controlled ovarian hyperstimulation than traditional parameters such as age, FSH, estradiol and inhibin-B.

Study design, size, duration: This is a single-arm uncontrolled clinical trial that was conducted between June 2018 and 2019. The study protocol was approved by the Institutional Ethics committee. Sixty women with the diagnosis of unexplained infertility who received controlled ovarian stimulation and ICSI treatment were enrolled. The inclusion criteria for women were age ≤ 38 years, normal ovulatory cycles and a body mass index (BMI) ranging from 18 to 28 kg/m2.

Participants/materials, setting, methods: This study was conducted at Minia Infertility center, Minia University. Follicular fluids (FF) were aspirated under transvaginal ultrasound guidance. The FF from the single dominant follicle of each patient (which is aspirated first) was separated. FF was stored at - *80* °C until assayed. AMH levels were measured by using an ultrasensitive enzyme-linked immunosorbent assay. Based on the median of cycles measured values; women were categorized as low and high FF-AMH groups.

Main results and the role of chance: The primary outcome of this study was fertilization rate, implantation rate, blastocyst development, embryo quality, chemical pregnancy and clinical pregnancy. Low FF-AMH group, compared to high FF-AMH group, showed a significant improvement with regards to percentage of top-quality oocytes (67.1±24.3% vs. 49.6 ± 30.3%, $p = 0.014$), FR (83.9±20.9% vs. 72.4 ± 21.4%, $p = 0.021$), IR (57.7% vs. 16.7%, $p = 0.001$) and CPR (57.57% vs. 16.67%, $p < 0.0001$). None of the remaining variables were significantly different between the two studied groups (p > 0.05). Further, FF-AMH value had a significant low correlation with follicular fluid estradiol (FF E2) (r = −0.409, p <0.001) and moderate correlation with clinical pregnancy (r = −0.618, p<0.001). FF-AMH pregnancy threshold was >1.75 ng/ml. Receiver operating characteristic (ROC) analysis showed a FF-AMH sensitivity of 73.1% in predicting CPR and a specificity of 85.3 %. The area under curve (ROC_{AUC}) was 0.715 ($p < 0.0001$).

Limitations, reasons for caution: The sample size was relatively small and does not reflect a wide variety of clinically seen cases such as women with ovarian cyst (> 3 cm in diameter), polycystic ovarian syndrome, endometriosis, or those with a history of previous ovarian surgery or endocrine disorders.

Wider implications of the findings: This study demonstrated that FF AMH levels were significantly lower in fertilized oocytes than in non-fertilized oocytes. FF-AMH is an adequate predictor of clinical pregnancy after ICSI; However further studies on a larger group of patients are needed to correlate its relation with ongoing pregnancy.

Trial registration number: not applicable

P-648 Does thyroid autoimmunity and more specifically anti-thyroglobulin antibodies affect IVF/ICSI outcome?

V. Uvin[1], M. De Brucker[1], P. Drakopoulos[1], K. Poppe[2], H. Tournaye[1], D. Unuane[3]

[1]UZ Brussel, Centre of reproductive medicine, Brussels, Belgium ; [2]Centre Hospitalier Universitaire Saint-Pierre, Endocrinology, Brussels, Belgium ; [3]UZ Brussel, Endocrinology, Brussels, Belgium

Study question: Does the thyroid autoimmune (TAI) status and especially anti-thyroglobulin antibodies affect IVF/ICSI outcomes of euthyroid patients?

Summary answer: Thyroid autoimmune status and more specifically anti-thyroglobulin antibodies do not affect the reproductive outcomes of euthyroid patients undergoing IVF/ICSI.

What is known already: The impact of TAI on fertility outcomes of euthyroid women undergoing IVF/ICSI has been controversial. In literature, in addition, TAI is predominately defined by elevated levels of thyreoperoxydase antibodies (TPO-Ab). The impact of anti-thyroglobulin antibodies (TG-Ab) on fertility outcome after IVF/ICSI remains unclear.

Study design, size, duration: This was a retrospective study including all euthyroid patients who underwent their first IVF/ ICSI cycle in a 6 month period at the center of reproductive medicine of the University Hospital Brussels. All patients were screened for both TPO-Ab and TG-Ab.

Participants/materials, setting, methods: Patients were categorized to four groups based on TAI status: TAI negative, only TG-Ab positive, only TPO-Ab positive and both TG- and TPO-Ab positive. The main outcome was fresh live birth rates (LBR). Secondary endpoints were positive human chorionic gonadotropin (hCG), early pregnancy loss and cumulative LBR. Cumulative LBR was defined as the delivery of at least one live-born infant (>24 weeks of gestation) in the fresh or in the subsequent frozen-thawed cycles.

Main results and the role of chance: In total, 464 patients were included in the analysis: 402 (86.6%) in the TAI negative group, 17 (3.7%) in the TG-Ab group, 15 (3.2%) in the TPO-Ab group and 30 (6.5%) in the TPO/TG-Ab group. Baseline characteristics such as age, body mass index, anti-Müllerian hormone (AMH) and antral follicle count (AFC) were similar between the four groups. Only thyroid-stimulating hormone (TSH) differed significantly [1.6 (0.7) vs. 2.4 (0.9) vs. 1.4 (0.8) vs. 1.8 (0.9), p value=0.005)]. Fresh LBR did not differ significantly between the four groups [131/402 (32.6%) vs. 3/17 (17.7%) vs. 6/15 (40%) vs. 8/30 (26.7%), p value=0.5)]. Similarly, positive hCG and early pregnancy loss rates were comparable between the four groups, while cumulative LBR did not show a statistically significant difference either [164/402 (40.8%) vs. 6/17(35.3%) vs. 6/15 (40%) vs. 11/30 (36.7%), p value =0.94]. Multivariate regression analysis indicated that after adjustment for relevant confounders the TAI status was not significantly associated with either fresh or cumulative LBR (p value = 0.24).

Limitations, reasons for caution: This is a large observational study based on retrospective data collection. Despite our robust methodological approach, the presence of bias related to the retrospective design cannot be excluded. Furthermore, the rarity of TG-Ab may preclude firm conclusions.

Wider implications of the findings: Our study adds further to the growing evidence that TAI (and especially TG-Ab) do not seem to affect the reproductive outcomes of euthyroid patients undergoing IVF/ICSI. These results should be confirmed in future prospective studies.

Trial registration number: not applicable

P-649 FF-MAS oxysterol related with hedgehog signaling pathway on folliculogenesis

S. ZIRH[1], E. Bahador Zirh[1], L. Karakoc Sokmensuer[1], G. Bozdag[2], S. Muftuoglu[1]

[1]Hacettepe University- School of Medicine, Histology and Embryology, Ankara, Turkey ;
[2]Hacettepe University- School of Medicine, Obstetrics and Gynecology, Ankara, Turkey

Study question: Does FF-MAS affect folliculogenesis via hedgehog cell signaling pathway?
Summary answer: The main effect of FF-MAS on folliculogenesis manifested by granulosa cell(GC) proliferation, is associated with the hedgehog pathway (HH) proteins SMO and GLI1.
What is known already: HH is one of the main cell signaling mechanisms effective on cell proliferation. It exerts its biological effects through signaling cascade that activated by smoothened(SMO) receptor consequently regulated glioma-associated oncogene(GLI) transcription factors. Follicular fluid–meiosis activating sterol(FF-MAS), which is a steroid derivative and present in follicular fluid, is involved in folliculogenesis. GC is critically important cell involved in follicular development during the ovarian cycle. Polycystic ovary syndrome(P-COS) defined as follicular development failure, lag of granulosa cell proliferation, and cystic follicles as a result, is one of the common endocrine disorders of today.
Study design, size, duration: Primary culture of granulosa cells from both PCOS (n=10) and healthy (male factor infertility)(n=10) woman undergoing IVF, were collected since 2018. The human immortalised granulosa cell line (hGL5, abm, Canada) were also used beside primary cell culture. Each GCs divide into four groups individually: FF-MAS, FF-MAS+cyclopamine, only cyclopamine and vehicle used for FF-MAS and cyclopamine. Cyclopamine was used for inhibiton of HH.
Participants/materials, setting, methods: Granulosa cells of women undergoing IVF, have isolated from follicular fluid at oocyte pick up in Hacettepe University Infertility Center. GCs (PCOS and control (male factor[MF] infertility)) were cultivated at least 3 passages after the isolation. ED50 dose of FF-MAS was maintained by WST assay. Hedgehog pathway mollecules(SMO, GLI1) were screened on granulosa cells from each group by using immunocytochemistry (immunofluorescence and immunoperoxidase) and cyto-ELISA. Mean fluorescence intensities evaluated in all micrographs by ImageJ 1.52K.
Main results and the role of chance: The ED50 dose of FF-MAS was determined as 10μM FF-MAS for 48h according to WST assays. Results were evaluated according to measurement of cell proliferation and viability in response to different doses of FF-MAS. Tests were performed in GCs of PCOS, MF and HGL5. Experiments carried out with 10μM FF-MAS for 48h according to maximum non-toxic dose and duration. All the GCs were assesed in terms of GLI1 and SMO by both IF, ICC. Hedgehog pathway was slightly inhibited with cyclopamine binding to signal transducer receptor SMO via canonical pathway. Indirect IF is widely considered for SMO-GLI1 proteins in all groups of granulosa cells screening with/without FF-MAS and cyclopamine.

The expression of GLI1 and SMO showed different flourescence intensities between groups. GLI1 and SMO were mostly expressed in all FF-MAS groups. The mean fluorescence intensities were compared in all groups by ImageJ analysis. A significant difference were obtained between quantitative reading of intensities in FF-MAS and FF-MAS+cyclopamine groups. Results of immunoperoxidase cyto-ELISA for GLI1 and SMO expressions of FF-MAS groups confirmed the IF analysis.

According to our findings, the highest expression was observed in FF-MAS groups especially in PCOS patients than MF group. Cyclopamine significantly suppressed the expression of proteins.
Limitations, reasons for caution: Since human cells were used in study and the cells of each patient do not exhibit the same characteristics, the lowest number of patient samples identified in the statistical power analysis were included in the study.
Wider implications of the findings: First time, the effect of FF-MAS via HH was evaluated on human GCs, which has been used in animal ART before. We have introduced a new approach to the literature on the molecular mechanisms of FF-MAS and to be considered as a new approach in human IVF treatments of PCOS.
Trial registration number: not applicable

P-650 Steroid levels in single follicular fluid: relation to ovarian stimulation protocol and destiny of the corresponding oocyte in assisted reproduction

E. Baldi[1], S. Marchiani[2], L. Tamburrino[1], F. Benini[3], M. Pallecchi[4], C. Bignozzi[3], A. Conforti[5], C. Alviggi[5], L. Vignozzi[4], G. Danza[4], S. Pellegrini[3]

[1]University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy ;
[2]Azienda Universitario Ospedaliera Careggi, Department Materno Infantile, Florence, Italy ;
[3]Centro Procreazione Assistita Demetra, none, Florence, Italy ;
[4]University of Florence, Department of Experimental Clinical and Biomedical Sciences, Florence, Italy ;
[5]University of Naples- Federico II, Department of Neuroscienze- Reproductive Science and Odontostomatology, Naples, Italy

Study question: Are steroid hormone levels in single follicular fluid related to ovarian stimulation (rFSH or rFSH+rLH) and to Assisted Reproduction Technique outcomes of the corresponding oocyte?
Summary answer: FF steroid levels were found different between the two protocols of ovarian stimulation. FF Progesterone levels predicts the attainment of fertilization in the FSH group.
What is known already: Conflicting results have been reported in studies concerning the relationship between steroid levels in FF and oocyte maturity or Assisted Reproduction Technique (ART) outcomes. Controversy may depend on the fact that most of these studies did not consider the destiny of individual oocytes and utilize immunoassay to measure steroid levels, which is affected by cross-reactivity and shows poor specificity and sensitivity.
Study design, size, duration: We enrolled in a prospective cohort study 41 couples undergoing ICSI cycles from November 2016 to August 2018. In 13 cases follicle stimulation was performed with recombinant r–FSH alone and in 28 women, with previous poor ovarian responsiveness, with r-FSH plus r-LH. 316 oocytes and the corresponding collected follicular fluids (FF) were included in the study. The destiny of each oocyte was followed during the entire cycle.
Participants/materials, setting, methods: Levels of six steroid hormones (progesterone (P), 17-OH-Progesterone (17-OH-P), Androstenedione (A), Testosterone (T), Estradiol (E1) and Estrone (E2)) were evaluated in the single FF by High Performance Liquid Chromatography coupled with tandem Mass Spectrometry (HPLC/MS-MS). Such levels were related to ovarian stimulation protocol and oocyte maturity, fertilization and quality of blastocysts. Clinical pregnancy, due to the limited number of achieved pregnancies of our cohort, was evaluated as explorative outcome.
Main results and the role of chance: Our study demonstrates that FF steroid levels vary among different follicles collected from the same woman and that the two ovarian stimulation protocols lead to differences in the steroid composition of FF, as 17-OH-P, A, E2 and E1 were significantly higher in FSH+LH group. In the latter group, P, 17-OH-P and E2 were more expressed in FF of follicles which yield a mature oocyte than in immature ones (median values (ng/ml): P, 7352.7 [IQR:4252.2-11378.2] vs. 4270.2 [IQR: 2466.4-8064.9]; 17-OH-P, 991.2 [IQR: 596.2-1620.4] vs. 571.4 [IQR: 352.6-1072.3]; E2, 396.0 [IQR: 238.5-551.4] vs. 245.9 [IQR: 165.3-385.6], $p<0.01$) and 17-OH-P was higher in FF of follicles which yield fertilized oocytes respect to non-fertilized oocytes (median values (ng/ml): 1076.6 [IQR: 663.7-1694.6] vs. 811.9 [IQR:362.3-1385.7], $p<0.05$). Moreover, P FF levels of the oocytes which achieved clinical pregnancy were significantly higher respect to non-pregnant (median values (ng/ml): 11136.9 [IQR: 9294.1-15275.5] vs. 8305.6 [IQR: 4688.3-11238.9], $p<0.05$). In the group stimulated only with FSH, we observed no differences in steroid levels for none of the analysed ART outcomes, except for P levels, which were found to be predicted of successful fertilization with an accuracy of 70.3±6.0%, $P<0.005$.
Limitations, reasons for caution: The low absolute number of embryos in the three quality categories and the paucity of women achieving pregnancy in the two groups may have limited the statistical power regarding the role of FF steroids in predicting embryo quality and pregnancy.
Wider implications of the findings: LH supplementation modifies the ovarian steroidogenic activity better resembling the physiological steroid pattern likely improving ovarian response. Our study does not support a direct association between FF steroid levels and ART outcomes, with the exception of Progesterone, which predicts with fair sensitivity and specificity the attainment of fertilization.
Trial registration number: not applicable

P-651 AMP-activated protein kinase (AMPK) controls dormancy of the primordial follicle pool in murine ovarian tissue

J. Nielsen[1], M. Amoushahi[1], E.H. Ernst[2], K. Lykke-Hartmann[1,3,4]

[1]*Aarhus University, Department of Biomedicine, Aarhus C, Denmark ;*
[2]*Herning Region Hospital, Department of Obstetrics and Gynaecology, Herning, Denmark ;*
[3]*Aarhus University Hospital, Department of Clinical Genetics, Aarhus C, Denmark ;*
[4]*Aarhus University, Department of Clinical Medicine, Aarhus C, Denmark*

Study question: Can inhibition of AMPK stimulate growth of primordial follicles in ovarian tissue?
Summary answer: AMPK signaling is an essential signaling pathway governing dormancy of primordial follicles and inhibition of AMPK enhances the number of activated follicles.
What is known already: Molecular signaling pathways including PI3K/AKT, AKT/mTOR, and HIPPO contribute to regulate the primordial-to-primary follicle transition in the mammalian follicle development. It is, however, widely acknowledged that we are only beginning to elucidate the molecular mechanisms maintaining dormancy and activation of primordial follicles. Recently, our team performed transcriptomic profiles of human primordial and primary follicles providing molecular clues towards which alterations in gene expression that dictate dormancy and activation of primordial and primary follicles. One promising gene candidate identified was *AMPK*; a kinase responsible for restoring cellular energy homeostasis.
Study design, size, duration: Whole murine ovaries were excised from female juvenile mice (C57BLxCBA F1) and randomly divided into two groups of equal size and grown in a primary three-dimensional culture system in culture medium supplemented with an inhibitor of AMPK (IHB) or without (NT). After five to seven days of culture, ovaries were collected.
Participants/materials, setting, methods: *In vitro*-cultured ovaries were collected for histological analysis to address the balance between follicular activation and quiescence after drug-exposure. Additionally, we addressed important parameters that can evaluate the follicle quality after drug-exposure. Towards this, we performed assays e.g. TUNEL, BAX-to-BCL-2 ratio, cytochrome c oxidase activation, and ROS generation. Furthermore, follicles from the drug-exposed and NT ovaries were mechanically isolated and cultured in an alginate hydrogel, culminating in meiotic competent oocytes.
Main results and the role of chance: We found that *in vitro* culture with an AMPK inhibitor for five days significantly increased the number of activated follicles (NT: 18.97%±1.017 *vs.* IHB: 39.73±1.28%, $p<0.0001$; n=17/15). As the level of activated AMPK were significantly decreased ($p<0.05$, n=18), and the activation of PI3K-AKT signaling was unaffected ($p<0.05$; n=18) by the drug-exposure, our results indicated that inhibition of AMPK can trigger follicular growth synergistically with PI3K-AKT signaling. Furthermore, we assessed the inhibitor's impact on oocyte quality parameters. We showed that inhibitor-exposure increased the survival rate by conducting a TUNEL analysis (number of apoptotic cells NT:7.48% *vs.* IHB:3.30, $p<0.05$, n=5-6) and the BAX-to-BCL-2 ratio were also significantly decreased after drug-exposure ($p<0.01$, n=12). Moreover, we showed that the activity of cytochrome c oxidase ($p>0.05$, n=12) and ROS generation ($p>0.05$, n=6) were not significantly affected by the drug-exposure. In line with these observations, we elucidated that follicles isolated from drug-exposed ovaries had the ability to release meiotic competent oocytes in response to an ovulating dose of human chorionic gonadotropin, with the same survival rate as the control-exposed ovaries (NT: 76.47% *vs.* IHB: 73.33%; n=34/30).
Limitations, reasons for caution: The study is for now limited to murine tissue and further studies are needed to assess if the murine findings are translatable into the human reproductive system. Moreover, the inhibitor has previously been reported to promote promiscuous AMPK-independent affairs, therefore the results must be interpreted with potential off-targets in mind.
Wider implications of the findings: This study furthers our understand on cellular signaling which dictate the balance between follicular activation and dormancy, and understanding the details of ovarian biomechanics may have substantial clinical implications.
Trial registration number: Not applicable

P-652 Automated quantification of the distribution of pre-antral ovarian follicles to facilitate accurate and unbiased counting

E. Bruun[1], M. Amoushahi[1], K. Lykke-Hartmann[2,3]

[1]*Aarhus University, Department of Biomedicine, Aarhus C, Denmark ;*
[2]*Aarhus University, Department of Biomedicine and Department of Clinical Medicine, Aarhus C, Denmark ;*
[3]*Aarhus University Hospital, Department of Clinical Genetics, Aarhus, Denmark*

Study question: We sought to improve and more precisely determine the follicle distribution of primordial, primary and secondary follicles in ovaries from juvenile mice by automated counting.
Summary answer: Fluorescent labeling of oocytes and quantification of follicles by automatic analysis software generates comparable data with conventional methods and ensures objective results.
What is known already: Knowledge of the number of ovarian follicles is of interest to investigators studying mammalian reproductive biology. One of the most widely used approaches for estimating the number of follicles in different developmental stages is based on histological sampling of the total ovarian mass followed by manual classification and counting using a microscope. This procedure is time-consuming and highly operator dependent. Improving the accuracy of the follicle classification and quantification will reduce time and produce more reliable results.
Study design, size, duration: In this pilot study, whole ovaries isolated from juvenile mice (7-8-days old) were used for quantification of the number of follicles in three different developmental stages; primordial, primary and secondary follicles. Ovaries were kept in organ culture for four days in basic medium in a well-insert culture system.
Participants/materials, setting, methods: Ovaries were fixed, processed, and embedded in paraffin. Samples were cut in 5-µm sections and stained with hematoxylin and eosin (H&E) or immunofluorescence (IF) staining. The number of follicles at three developmental stages was either counted manually using an inverted research microscope or automatically using high-content image analysis software (MetaXpress). The follicle distribution of every 5th section of each ovary was counted. Germ-cell marker MVH was used to visualize oocytes for IF.
Main results and the role of chance: IF staining results showed a strong MVH signal in oocyte cytoplasm of follicles in all three developmental stages present in the cultured juvenile mouse ovaries. The average distribution of follicles classified and counted manually was 83% (SD 3%), 13% (SD 2%) and 4% (SD 1%) among primordial, primary, secondary follicles, respectively. The preliminary customized analysis module in MetaXpress was programmed to recognize all MVH-positive follicles with a certain intensity and with a visible nucleus. Only about 5% of the visible follicles were not recognized by the analysis module. Since stage-specific IF markers were still not identified, the preliminary results of the analysis module were based on the number of follicles rather than classification of the follicles. By setting a diameter range for the different follicular stages, the analysis software discovered 10% (SD 3%) more follicles in the primordial stage compared to manual counting. This method is a way of eliminating workload and subjectivity in microscopic image analysis, however, human assessment of the health of the ovary is always important.
Limitations, reasons for caution: The main limitations of this study are the lack of stage-specific markers for the different pre-antral developmental stages. Additionally, this method will not provide the total number of follicles of the whole ovary, but the method can be used as an estimation of the effect of an experimental intervention.
Wider implications of the findings: Automated image analysis provides an important advantage by significantly reducing manual counting variability. IF methods also efficiently enhance the visibility of primordial follicles that are difficult to see and ensures accurate recording of the number of follicles. Focus on finding stage-specific markers would further improve the classification of follicles.
Trial registration number: Not applicable

P-653 Human oocytes and granulosa cells from primordial and primary follicles revealed expression patterns of genes encoding Toll-like receptors (TLRs)

G. Saritas[1], E.H. Ernst[2], M. Amoushahi[1], K. Lykke-Hartmann[1,3,4], T. Wenzel Kragstrup[1], A.S. Sørensen[1]

[1]*Aarhus University, Department of Biomedicine, Aarhus C, Denmark ;*
[2]*Herning Region Hospital, Department of Obstetrics and Gynaecology, Herning, Denmark ;*
[3]*Aarhus University Hospital, Department of Clinical Genetics, Aarhus N, Denmark ;*
[4]*Aarhus University, Department of Clinical Medicine, Aarhus C, Denmark*

Study question: Does the early hormone-independent, primordial-to-primary follicle development involve inflammatory-like responses mediated through TLRs?
Summary answer: The hormone-independent transition from primordial to primary follicles in human folliculogenesis revealed oocyte- and granulosa cell-specific expression patterns and biological functions of TLR3 and TLR4.
What is known already: Later stages of follicle development and ovulation, encompassing the maturation of secondary to antral/preovulatory follicles, involves inflammatory-like responses similar to the innate immune-related cell surveillance system including: inflammation, rupture (or ovulatory) event and reparation. Cumulus oocyte complex, which appears in antral follicles, secretes luteinizing hormone and amphiregulin inducing expression of immune-related receptors as TLRs. Murine granulosa and cumulus cells exposed to these hormones revealed gene expression levels of various immune components, e.g. TLR2, TLR4 and TLR9. The impact of immune system on the primordial-to-primary follicle transition remains unknown which could clarify molecular mechanisms protecting these early follicle stages.
Study design, size, duration: Expression data of *TLR* transcripts was extracted using bioinformatic tools from previous global RNA transcriptome studies in human Laser Capture Microdissection (LCM)-isolated oocytes and granulosa cells from primordial (n= 539) and primary (n= 261) follicles. Ovarian tissues were donated by three women undergoing ovarian removal surgery for cryopreservation before gonadotoxic treatment of non-gynecological cancer. As a proof of concept, intrafollicular localization and functionality of selected TLRs investigated by immunohistochemical and interleukin 6 (IL-6)-specific ELISA analyses.
Participants/materials, setting, methods: qPCR was performed on LCM-isolated oocytes and granulosa cells from two independent patient samples to confirm *TLR* transcriptome data. Intrafollicular localization of TLR3 and TLR4 were studied in two patient samples using immunohistochemistry with anti-TLR3 and -TLR4 antibodies. For ELISA analysis, supernatants were extracted from juvenile murine ovaries *in vitro* cultured without or either with polyinosinic:polycytidylic acid (poly I:C), or with lipopolysaccharides (LPS) (known as ligands for TLR3 and TLR4, respectively).
Main results and the role of chance: Among other *TLR* transcripts, *TLR3*, *TLR4* and *TLR5* were differentially and stage-specific consistently expressed genes (SSCEG) in both/either oocytes and/or granulosa cells from primordial and primary follicles. Inconsistent *TLR5* expression across the three patient samples was the cause to exclude from this study. The qPCR analysis on *TLR3* transcript aligned with the transcriptome expression levels indicating highly upregulation only in granulosa cells from both follicle stages. Immunohistochemistry indicated a halo-shaped TLR3 staining in the interspace between surrounding granulosa cells and the oocyte in primordial and primary follicles, respectively. Primary follicles showed a more intense TLR3 staining correlating with the significant mRNA levels from the transcriptome data. TLR4 staining indicated presence in both the oocyte and granulosa cells from primordial and primary follicles, however a weaker staining in primordial follicle than observed for TLR3 in primordial follicle. ELISA revealed higher IL-6 production in ovarian supernatants treated with LPS than the level in untreated and poly I:C-treated supernatants. These TLRs are clearly present in the earliest hormone-independent follicle stages in human ovary which may have a potential defensive role (especially in murine ovaries).
Limitations, reasons for caution: This is a descriptive study of *TLR* transcripts in human ovary supported with their biological functions in murine ovary. The number of human samples was limited which eliminates the considerations of the natural biological variance in the samples according to the experimental design.
Wider implications of the findings: This study has indicated, for the first time, presence and functional role of pattern recognition receptors as *TLR3* and *TLR4* in human oocytes and granulosa cells from the hormone-independent primordial and primary follicle stages.
Trial registration number: Not applicable

P-654 Serum selenium level in women with idiopathic premature ovarian insufficiency: A case-control study

B. Sadeghzadeh[1], M. Bazrafkan[2], P. Shahabi[3]

[1]Tabriz university of Medical sciences, midwifery Department, Tabriz, Iran ;
[2]Reproductive Biotechnology Research center- Avicenna Research Institute, Reproductive Biology, Tehran, Iran ;
[3]Tabriz University of Medical Sciences, Physiology, Tabriz, Iran

Study question: What is the relationship between selenium level and premature ovarian insufficiency?
Summary answer: Patients with abnormal low level of selenium have low ovarian reserve.
What is known already: Selenium is a co-factor of the antioxidant enzymes responsible for prevention of ROS production. Glutathione peroxidases (GPxs) are the main selenoprotein enzymes associated with reproductive and pregnancy processes.The importance of serum selenium assessment in infertile women with POI lies in the fact that antioxidants, including selenoprotein enzymes such as GPxs, are highly sensitive to changes in selenium status and decrease significantly with selenium deficiency. The present study was therefore conducted to assess serum selenium level and GPx activity (main objectives) and nutrition status in terms of selenium intake (secondary objective) in infertile women with POI compared to healthy fertile women.
Study design, size, duration: Study design: Case-control study.
size: Case group consisted of 29 infertile women with idiopathic POI, amenorrhea and FSH >40 mIU/ml and three occult OI with oligomenorrhea and AMH <0.3 ng/ml. Control group cosisted of 31 healthy fertile women matched with the case group in terms of age and BMI.
Duration: from August 2018 to Feb 2019.
Participants/materials, setting, methods: Case group consisted of 29 infertile women with premature ovarian insufficiency (POI), amenorrhea and FSH >40 mIU/ml.
Infertility referral centers and tertiary hospital.
methods: The serum selenium level was measured by an atomic absorption spectrophotometer, and plasma GPx activity was measured by a Glutathione Peroxidase Activity Assay Kit after about 12 hours of fasting.
Main results and the role of chance: There was a significant reduction in the serum selenium level in the case group compared to the control group (Adjusted Mean Difference (AMD) = -15.1 µg/ml, 95% CI: -24.8 to -5.3). The plasma GPx activity was lower in the case group compared to the control group, although not significantly (AMD = -67.0 U/ml, 95%CI: -194.5 to 60.3).
Limitations, reasons for caution: present study limitations were selenium measurement fee.
Wider implications of the findings: This study showed a significant decline in selenium status in infertile women with POI. It could weaken the selenium-dependent antioxidant defense against reactive oxygen species and may lead to POI.
Trial registration number: IRCTID: IRCT2016041002731 1N6

P-655 Bilirubin in follicular fluid: a biochemical signature of female infertility

R. Pallisco[1], G. Bilotta[2], I. Listorti[2], P. Bilotta[3], G. Lazzarino[4], B. Tavazzi[5], T. Capozzolo[3], A. Guarino[3], G. Lazzarino[6], R. Mangione[7]

[1]Alma Res fertility center, Laboratory of Embriology, roma, Italy ;
[2]Alma Res Fertility Center, Laboratory of andrology and embriology, Rome, Italy ;
[3]Alma Res Fertility Center, Obstetrics and Gynecology, Rome, Italy ;
[4]University of Catania, Department of Biomedical and Biotechnological Sciences-Division of Medical Biochemistry, Catania, Italy ;
[5]Catholic University of Rome and Fondazione Policlinico Gemelli, Institute of Biochemistry and Clinical Biochemistry, Rome, Italy ;
[6]Saint Camillus International University of Health Sciences, Biochemistry, Roma, Italy ;
[7]Catholic University, Institute of Biochemistry and Clinical Biochemistry, Rome, Italy

Study question: To assess whether bilirubin in follicular fluid (FF) of fertile females differs from infertile. To correlate FF bilirubin concentration with in vitro fertilization (IVF) outcomes.
Summary answer: Despite normal plasma levels, FF bilirubin concentrations in infertile females are higher than in fertile females. FF bilirubin concentrations negatively correlate with main IVF measures.
What is known already: Successful in vitro fertilization (IVF) are unexplainably low (about 32%), not only in females with full-blown pathologies, but also in those known as idiopathic. Studies indicated that the composition of FF of infertile female may show altered levels of proteins, lipids, hormones respect to values found in fertile females. Such changes were associated to endometriosis, polycystic ovarian syndrome (PCOs), diminished ovarian reserve (DOR). To date, no studies have been dedicated either to determine potential differences in the FF concentration of bilirubin in fertile and infertile females or to evaluate possible correlations with main IVF outcome measures.

Study design, size, duration: Cross-sectional study in seven female groups: 1) controls (n = 34); 2) endometriosis (n = 19); 3) PCOs (n = 12); 4) DOR (n = 31); 5) age related DOR (n = 55); 6) unexplained infertility (n = 16); 7) genetic disorders (n = 11). Patients were enrolled over a period of 16 months, from September 2018 to January 2020. As inclusion criteria, patients should have total plasma bilirubin within normal physiological range (3.4-17.1 µmol/l).
Participants/materials, setting, methods: We enrolled a total of 178 patients being tested for infertility. All patients underwent oocytes retrieval and 135 of them were submitted to conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). FF was processed by high performance liquid chromatography (HPLC) to quantify bilirubin concentration. For all patients were counted the number of cumulus-oocyte complexes (COCs), MII oocytes, zygotes and blastocysts. FF analysis was performed in blind with respect to IVF outcome measures.
Main results and the role of chance: FF total bilirubin concentration was 1.20 ± 0.42 mmol/l in controls (n = 34) and 3.07 ± 1.20 mmol/l in pooled infertile patients (+256%, $p < 0.001$). Total bilirubin in patients with endometriosis, PCOs, DOR, age related DOR, unexplained infertility and genetic disorders was, respectively 2.34 ± 0.80, 2.74 ± 0.84, 2.99 ± 0.74, 2.66 ± 0.93, 3.18 ± 0.90, 4.77 ± 1.90 mmol/l FF (values of all groups were significantly different from those of controls, $p < 0.001$). To evaluate whether increase in FF total bilirubin might affect IVF outcome, we pooled all patients into a single group (n = 178) and calculated the Pearson's correlation coefficients of bilirubin (y) and number of MII oocytes (fx); bilirubin (y) and number of zygotes (fx); bilirubin (y) and number of good quality blastocysts (fx). Increase of FF total bilirubin negatively correlated with the number either of mature oocytes ($r = -0.348$, $t = -4.876$, $p < 0.001$), or zygotes ($r = -0.247$, $t = -2.959$, $p < 0.005$), or blastocysts ($r = -0.251$, $t = -3.033$, $p < 0.005$). Results strongly suggest that increase FF total bilirubin levels are detrimental for good oocyte quality and embryo development.
Limitations, reasons for caution: At present, the main limitation of this study is the lack of data on pregnancy rate that should better allow to understand the role of FF total bilirubin in infertility and IVF success rate.
Wider implications of the findings: If reinforced by the data of pregnancies and newborns, findings of the present study might represent a significant advancement in IVF treatment. Measuring total bilirubin in FF might be helpful in selecting the best oocytes for IVF/ICSI procedures, thereby possibly leading to a significant increase of IVF success rate.
Trial registration number: not applicable

P-656 Is there an effect of late-follicular phase elevated serum progesterone on the embryo quality?

F. Van Wynsberge[1], I. De Croo[1], F. Vandekerckhove[1]

[1]UZ Gent, Department for reproductive medicine, Gent, Belgium

Study question: Does late-follicular phase serum progesterone during ovarian stimulation have an effect on embryo quality on day 3 and day 5?
Summary answer: Late-follicular phase elevated serum progesterone does not have an influence on the embryo utilization rate.
What is known already: Ovarian stimulation promotes the production of progesterone which, when elevated during the follicular phase, has been demonstrated to have deleterious effect on the endometrium and IVF outcome.
Study design, size, duration: Retrospective study analysis of all IVF/ICSI cycles between July 2015 and June 2018. A total of 1456 unique cycles were included. The primary outcome was embryo utilization rate (number of embryos transferred and cryopreserved/number of zygotes). Secondary outcomes were the number of oocytes, the fertilization rate, the percentage of good quality embryos on day 3 (at least 7 cell stage), and the percentage of blastocysts on day 5.
Participants/materials, setting, methods: Serum progesterone levels on the day of ovulation triggering were analysed as a continuous variable. All IVF/ICSI cycles were included in the analysis, using a multivariate regression to account for potential confounders (female age, AMH concentration, BMI, down regulation protocol, infertility factors and IVF or ICSI).
Main results and the role of chance: The average female age was 34.5 ± 5.0 years and the number of oocytes retrieved was 11.1 ± 6.3. The mean number of oocytes retrieved significantly increased with an increase of progesterone of 0.1 ng/ml. The percentage of embryos with at least 7 cells on day 3 was not affected by an increase of progesterone on the day of ovulation triggering. The percentage of blastocysts on day 5 significantly decreased with an increase of progesterone of 0.1 ng/ml ($p=0.002$). A multivariate regression analysis accounting for confounders confirmed this effect on the percentage of blastocysts on day 5 ($p=0.008$). It showed no correlation between late-follicular phase elevated serum progesterone and embryo utilization rate ($p=0.126$).
Limitations, reasons for caution: This study is limited by its retrospective design. A variation in the time interval between the administration of medication (gonadotrophins, antagonist) and the blood test for determination of progesterone levels can lead to different results.
Wider implications of the findings: We need larger data sets before we can definitively rule out that elevated progesterone levels at the late-follicular phase have no effect on any stage of embryonic development.
Trial registration number: EC/2018/0818)

P-657 Examining the effect of nicotine on a mouse model of the ovarian reserve

S. Idrees[1], M. Fenwick[1]

[1]University of Sheffield, Reproductive and Developmental Medicine, Sheffield, United Kingdom

Study question:
Does nicotine, or the metabolite cotinine, or the combination of both have an effect on the development of small follicles in the mouse ovary?
Summary answer:
Small follicles were reduced in size when exposed to nicotine *in vitro*; however, exposure to cotinine caused no further effect on follicle size.
What is known already: Over the past decade, there has been a dramatic shift in the number of people who have exchanged tobacco-based cigarette smoking for less harmful alternatives such as electronic cigarettes. However, users are still exposed to variable levels of nicotine, and the metabolic by-products of this stimulant. Nicotine exerts its effects by binding to nicotinic acetylcholine receptors (nAChRs) and we have previously shown that specific nAChRs are expressed in small follicles in the mouse ovary; therefore, we aimed to analyse the effect of nicotine and cotinine on the ovarian reserve.
Study design, size, duration:
Day 4 mouse ovaries densely populated with small follicles (primordial, transitional and early growing) were cultured for 7 days and exposed to nicotine, cotinine, or a combination of both using concentrations derived from published serum levels from e-cigarette users. Nicotine: 0 (control), 5 (low), 15 (medium) or 45 (high) ng/ml (5-7 ovaries/ group). Cotinine: 0 (control), 12 (low), 60 (medium) or 300 (high) ng/ml (8-10 ovaries / group).
Participants/materials, setting, methods: Ovaries from wild-type C57/Bl6 mice were dissected and cultured for 7 days in 6 well transwell plates containing membrane inserts. For morphological assessment of follicle development ovaries were fixed, sectioned and immunofluorescently labelled with antibodies DDX4 (green) and SMAD2/3 (red) to clearly identify oocytes and granulosa cells, respectively.Follicles were classified by stage and measurements of oocyte, follicle size and granulosa cell area were determined from high-resolution confocal images using imageJ.
Main results and the role of chance: Mean follicle size was reduced in primordial and transitional follicles with all concentrations of nicotine ($P<0.05$). In early growing follicles, a small, but significant reduction in size was also detectable in the high nicotine group only ($P<0.05$). These effects were mainly attributable to a reduction in mean size of the granulosa cell compartment, rather than mean oocyte size. By comparison, cotinine had no observable effect on overall follicle, oocyte or granulosa cell compartment size, regardless of concentration used ($P>0.05$). When ovaries were exposed to nicotine and cotinine in combination, mean follicle size and granulosa cell compartment size were reduced in primordial follicles in the medium and high groups only ($P<0.05$); however, no size differences were observed in transitional and early growing follicles with any concentration.
Limitations, reasons for caution: A single endpoint was evaluated after short-term culture and therefore the effects on reproductive outcomes are

unknown. This is also an *in vitro*study on mouse tissues, which may differ from human physiology.
Wider implications of the findings: Preliminary findings from this study suggest that nicotine alone can cause subtle developmental effects on the small follicles that make up the ovarian reserve. This raises further questions about the impact of an increasing trend for consumption of e-cigarettes, on female fertility.
Trial registration number: Not applicable

P-658 Correlation between 3 qualitative markers of ovarian function with IVF outcomes and embryo euploidy. Analysis of 4947 patients

M.I. Riqueros Arevalo[1], M. Martín Salat[1], M. Esbert Algam[1], J. Peñarrubia Alonso[1], N. Garrido Puchalt[2], A. Ballesteros Boluda[3]

[1]IVI Barcelona, IVF Laboratory, Barcelona, Spain ;
[2]IVI Fundation, IVI Fundation, Valencia, Spain ;
[3]IVI Barcelona, Reroductive Medicine, Barcelona, Spain

Study question: Are Ovarian Sensitivity Index (OSI), Follicular Output Rate (FORT) or Follicular Sensitivity Index (FSI) related to IVF outcomes and embryo euploidy?
Summary answer: Gestational IVF outcomes are linked to both OSI's and FORT's levels, while embryo euploidy depends on OSI.
What is known already: Whereas regulatory mechanisms determining the extent of sensitivity of antral follicles to FHS remains to be elucidated, the correct response of antral follicles (AF) to gonadotropins and a high quality oocytes results in good outcomes after IVF/ICSI.

It is well known that maternal age is strongly correlated with euploidy rate, and recently, three ovarian biomarkers (OSI, FORT and FSI) have also been proposed as good predictors of clinical outcomes. However, to date, the literature about them is still scarce and there is not any study comparing these three biomarkers with the embryo euploidy status.
Study design, size, duration: This is a retrospective multicentric study including 4947 patients that underwent IVF cycles with their own fresh oocytes in 10 ART clinics (part of a single network) between January 2018 and December 2019. Patients were split into two groups (A: non-PGT-A cycles; B: PGT-A cycles) and were analysed individually correlating the three biomarkers of ovarian responsiveness with gestational results in the first group, and with embryo euploidy in the second group.
Participants/materials, setting, methods: Group A included 1795 patients [mean age: 35.15(±3.62), BMI: 23.21(±4.08)] and group B was constituted by 3152 patients [mean age: 38.69(±3.44), BMI: 23.46(±4.11)].

According to the literature, the biomarkers were obtained applying the following formulas: FORT= (number of pre-ovulatory follicles on hCG day x 100)/ AF count at baseline, OSI= (total administered rFSH dose/ number of oocytes at OPU) x 1000 and FSI= (pre-ovulatory follicles on hCG day x 10000)/(AF count x total FSH dose).
Main results and the role of chance: To assess the correlation between the 3 ovarian biomarkers and IVF outcomes in group A, univariate logistic regression models were applied. Interestingly, we found a statistically significant correlation between OSI and clinical pregnancy (OR=1.006, p<0.001), ongoing pregnancy (OR=1.004, p=0.008) and livebirth (OR=1.003, p=0.047). The two other biomarkers (FORT and FSI) were not correlated.
Poisson regression models were used to assess the relationship between the embryo ploidy status and the biomarkers studied. The number of euploid embryos was analysed by controlling the total number of informative biopsied embryos. In the univariate models, both the OSI (p<0.001) and the FORT (p=0.003) were statistically significant, while the FSI (p=0.187) was not.

We created a multivariate regression model in which all three biomarkers were included as explanatory variables, and the patient's age was added as a control variable. We used the Akaike information criterion in both directions as a method of variable selection and both age (p<0.001) and OSI (p=0.036) proved to be statistically significant in the final optimal model. For this model, the estimated coefficients for age and OSI were 0.0697 and 0.0018 respectively, so we associated a direct relationship between the euploidy rate and the OSI.
Limitations, reasons for caution: Retrospective design of the study.
Wider implications of the findings: To our knowledge, this is the largest multicentric study aiming to correlate OSI, FORT and FSI with both gestational and embryo aneuploidy rates.

Patients with lower OSI and FORT should be counseled to undergo PGT because they are more prone to have aneuploid embryos leading to lower gestational rates.
Trial registration number: not applicable

P-659 Comparison between the outcome of consecutive versus separate distant IVF cycles in poor responders

M. Simeonov[1], O. Sapir[1], H. Lande[1], A. Ben-Haroush[1], G. Orona[1], E. Shlush[1], E. Altman[1], A. Wertheimer[1], T. Shochat[2], Y. Shufaro[1]

[1]Rabin Medical Center – Beilinson Hospital, Infertility and IVF Unit- Helen Schneider Hospital for Women, Petach Tikva 4941492, Israel ;
[2]Rabin Medical Center – Beilinson Hospital, Medical Statistics, Petach Tikva 4941492, Israel

Study question: Is it better to start an additional IVF cycle in poor responders immediately after an unsuccessful cycle, or to perform them at a time distance?
Summary answer: In poor responders, the difference in the number of oocytes and embryos is higher if the cycles are performed consecutively rather than at a distance
What is known already: Different protocols and treatments adjustments have been attempted in order to improve the ovarian response in poor responders without proven superiority. It is unclear if there is a difference between immediate consecutive and separate ovarian stimulations and oocyte pickups in this population
Study design, size, duration: : A cohort of all fresh IVF-ET cycles performed in a referral IVF unit between 2001-2019 in patients producing £4 oocytes following high dose (³300IU/d) FSH stimulation without achieving an ongoing pregnancy, who had a second pickup within 180 days. Patients who had surplus embryos cryopreserved in the first cycle were excluded. If more than two pickups were performed in a patient, only the first 2 cycles, meeting the criteria, were included in the cohort
Participants/materials, setting, methods: The cycle pairs were divided into two groups based on whether the second pickup was consecutive (performed within 45 days from the first one, group 1; 143 cases) or if at least one full menstrual cycle (46-180 days) passed between the pickups (group 2; 489 cases). The clinical and embryological data of both IVF cycles was compared between the groups
Main results and the role of chance: All the clinical basic and treatment characteristics did not differ between groups 1 and 2. Compared to the first cycle, oocyte yield in the second cycle improved by 0.29±1.54 in group 1, while in group 2 the difference in oocyte yield was -0.09±1.45 (p< 0.005). The difference in the number of embryos available for transfer between the first and second cycles was in 0.22±1.18 for group 1 and -0.06±1.3 for group 2 (p<0.019). There was no difference between groups 1 and 2 in the ongoing pregnancy rate following the second cycle
Limitations, reasons for caution: Although these results suggest that consecutive stimulations in low responders offer an advantage in the number of aspirated oocytes and available embryos in comparison with separate stimulations, this did not translate into a significant advantage in the ongoing pregnancy rate in a cohort of this size
Wider implications of the findings: Administration of high dose FSH might be beneficial for follicular recruitment also in the cycle to follow. Although this effect is modest in low responders, it might be beneficial for this patient population in which every aspirated oocyte counts. Cycle postponement under those circumstances was detrimental
Trial registration number: not applicable

P-660 Endocrine profile in Polycystic Ovary Syndrome (PCOS) patients performing frozen thawed embryo transfer in artificial cycle is significantly correlated with the ongoing pregnancy rate (OPR).

A.R. Oumeziane[1], N. Chabane[1], N. Tazairt[1], S. Barbara[1], B. Nadjia[2], F. Nanouche[3], A. Lachehab[1], S. Chemoul[1], S. Mouhoub[1], R. Bourihane[1], F. Ould el hocine[4], S. Aithadi[5], P.L. Devroey[6]

[1]tiziri ivf center, Gynecology-IVF unit, Algiers, Algeria ;
[2]tiziri ivf center, embryology unit, Algiers, Algeria ;
[3]tiziri ivf center, ivf unit, Algiers, Algeria ;

[4]tiziri ivf center, hormonology-unit, Algiers, Algeria ;
[5]tiziri ivf center, hormonogy unit, Algiers, Algeria ;
[6]none, none, brussels, Belgium

Study question: Is endocrine profile in PCOS patients, performing a single frozen thawed embryo transfer in hormonal replacement therapy (HRT) correlated with pregnancy outcomes?
Summary answer: In PCOS patient performing a single frozen thawed embryo transfer, LH and progesterone concentrations either FSH/LH ratio are strongly correlated with pregnancy outcomes.
What is known already: Since the development of vitrification embryo cryopreservation became wildly used (Rienzi 2016).To date it is not clear to recommend a specific regimen for patients who undergo frozen thawed embryo transfer (Ghobara 2017,Groenewoud 2018). HRTcycle or natural cycle are equally used .Recently it has been demonstrated that serum progesterone (P) concentration at the day of embryo transfer could negatively interfere with OPR in HRT cycle (Brady 2014,Yovitch2015,Labarta 2017).The duration of exposition to estrogen as well as the estradiol (E2) serum concentration and the route of administration of these hormones in HRT cycle are still in debate(Kahraman2018, Devine2018,Wen He2017).
Study design, size, duration: It is an observational prospective cohort study, conducted from April 1 2018 to December 31 2019, including 121 PCOS and normo-ovulatory patients, who performed a single frozen thawed embryo transfer in an artificial cycle .Patients with uterine abnormalities, auto immune pathology and implantation failure, were excluded. Chi^2, Student and Wilcoxon tests,either ROC curve, were applied for the statistical analysis .

The multivariate analysis regression was used, adjusting potential confounders to the OPR.
Participants/materials, setting, methods: 121 patients under 37 years performed a single frozen thawed embryo transfer.Only a good quality blastocyst at day 5 was replaced. Endometrium preparation was done with oral estrogen and vaginal progesterone .E2,LH and P were analyzed three times: on day two of the cycle,the day of introducing progesterone and the day of embryo replacement. 27 endometrium biopsy for histological evaluation were done in a previous simulated cycle.

Setting of the study: TIZIRI IVF Center Algeria.
Main results and the role of chance: Out of 121 patients, 39 suffered from PCOS (groupe1) and 82 were normo -ovulatory ones(groupe2). In the group 1,the threshold value of baseline LH up to 11.05IU/ml determinate by ROC curve was negatively correlated to OPR 2,5% vs 25,6% P= 0,018 with an area under curve(AUC) of 0.73. FSH/LH ratio below 0,645 was also a predictor of reduced OPR 5% vs 23% P= 0,016,AUC 0,79.Low serum progesterone the day of embryo transfer less than 12,5ng/ ml was associated with a significant diminished OPR 5% vs 23%p=0,028 ,AUC of 0,64. On the other hand in the group 2 the baseline LH level, the FSH/LH ratio, the progesterone at the day of embryo transfer are not correlated to the ongoing pregnancy rate. In this group the lowest OPR 9% vs 28% P=0,021, was associated with an endometrium thickness<8,6mm.After multivariate logistic regression, age, FSH/LH ratio and P on the day of transfer remained significant predictors for OPR .Whereas, the histologic evaluation of luteal phase endometrial receptivity didn't show any correlation with the OPR.
Limitations, reasons for caution: The pathogenesis of PCOS patients is complex; moreover the hormonal disorders of the PCOS could be link to an impaired pregnancy outcome.
Wider implications of the findings: The Polycystic Ovary Syndrome patients should benefit from a different regimen of endometrium preparation regarding to their specific endocrine profile ,while in normo-ovulatory patients, the endocrine profile didn't interfere with ongoing pregnancy.
Trial registration number: none

P-661 Gene Expression of Steroid Sulfatase and Transporter OATP2B1 is increased In Human Granulosa Cells from Women with Diminished Ovarian Reserve in IVF cycles

K.A. Sequeira Alvarado[1], S. Henriquez[1], P. Kohen[1], A. Fuentes[1], A. Tapia-Pizarro[1], P. Cespedes[1], A. Godoy[1], L. Devoto[1]

[1]Maternal and Child Research Institute, Faculty of Medicine- University of Chile, Santiago, Chile

Study question: Are transcript levels for Steroid sulfatase (STS) and organic anion transporting polypeptide (OATP)2B1 different in granulosa cells from older patient with diminished ovarian reserve vs normo-responder women?
Summary answer: Women with decreased ovarian reserve (POSEIDON 4) have higher transcript levels for STS and OATP2B1 in granulosa cells compared with women with normal ovarian response.
What is known already: Increasing evidence suggest that decreased circulating levels of dehydroepiandrosterone sulfate (DHEA-S) is associated with women with poor ovarian response to controlled ovarian hyperstimulation (COH). The uptake of DHEA-S is facilitated by the trans-membrane organic anion-transporting polypeptide OATP2B1. Once in the cytoplasm, STS enzyme catalyzes its transformation to DHEA. We hypothesize that if DHEA-S supply is important for ovarian function, in patients with diminished ovarian reserve, transcript levels for OATP2B1 and STS might be differentially expressed in granulosa cells form women of POSEIDON group 4 compared to controls.
Study design, size, duration: Prospective study which included 23women who had a cycle of in vitro fertilization at the Institute of Maternal and Child Research (IDIMI, Chile) between November 2017 and April 2019. Women who had Anti-mullerian hormone (AMH) <1.2 ng/dL and older than 35 years, met the criteria for low ovarian reserve POSEIDON 4 (n=13). The control group (n=10) included women of <30 years who had more of 12 oocytes picked-up after COH.
Participants/materials, setting, methods: Participants were treated using a gonadotropin-releasing hormone antagonist protocol for COH. We determine the gene expression (mRNA) by RT-PCR real time and the cellular distribution of sulfatase and the OATP2B1 transporter by immunofluorescence. In addition, the determinations of mRNA were related to age, AMH concentration, antral follicle count (AFC), DHEA-S and DHEA, in serum and in follicular fluid.The significance of the in vivo results was determined using Student's t-test.
Main results and the role of chance: As expected, subjects in group 4 of the POSEIDON classification had more age (38 ± 4,01 vs 25 ± 3,27 p< 0.05) and lower AMH levels (0.61 ± 0.05 vs. 2.29 ± 0.72 ng/dL p< 0.05) and AFC (4.33 ± 0.09 vs. 10.21 ± 2.45 p< 0.05). DHEA-S (383,62 ± 90,07 vs. 170,25 ± 79,04 µg/dlp< 0.05) DHEA (113,44 ± 47.23 vs 168.12 ± 38.63 µg/dlp= 0.06). The POSEIDON 4 women have significantly more expression of mRNA of STS and OATP2B1. (n=13, p< 0.05)
Limitations, reasons for caution: Caution is warranted due to the limited sample size of the study.
Wider implications of the findings: Our results suggest that up-regulation of STS and OATP2B1 in granulosa cells from women in POSEIDON group 4 could be a compensatory mechanism to overcome the decreased circulating levels of DHEA-S possibly required as substrate for intraovarian generation of DHEA.
Trial registration number: not applicable

P-662 The strategy to improve the number of competent embryos in carriers of translocation

J. Liss[1],[2], A. Knight[1], K. Lukaszuk[1,3,4]

[1]Invicta, Fertility and Reproductive Centre, Gdansk, Poland ;
[2]University of Gdansk, Department of Medical Biology and Genetics, Gdansk, Poland ;
[3]Medical University of Gdansk, Department of Obstetrics and Gynecological Nursing- Faculty of Health Sciences, Gdansk, Poland ;
[4]Medical University of Warsaw, Department of Gynecological Endocrinology, Warsaw, Poland

Study question: To evaluate the efficacy of double stimulation in terms of competent embryos in carriers of translocation.
Summary answer: Shorter time of oocyte recruitment can help increase the number of balanced blastocysts.
What is known already: The theory of a multicyclic development of follicles during the same menstrual cycle allows to collect the highest number of oocytes in the shortest possible time. Recent evidence indicates that embryos from this type of stimulation have similar developmental potential and euploidy rate compared to traditional ovarian stimulation. Translocation carriers have elevated numbers of unbalanced gametes. Reduced fertility may in part be the result of formation of a quadrivalent or trivalent structure during meiosis. It is important to know if the shorter time of oocyte recruitment can help to improve the number of balanced embryos.
Study design, size, duration: This was a retrospective case-control study and consisted of 35 women (median age 34 years, range 26-41), carrier of

translocations who were undergoing IVF treatment with PGT-SR with double ovarian stimulation in the follicular (FPS) and luteal phase (LPS) between November 2017 and October 2019 at INVICTA Fertility Centre, Poland.
Participants/materials, setting, methods: A total of 477 MII were derived (240 FPS and 237 LPS) and 149 blastocyst (68 FPS and 81 LPS) were evaluated with the NGS protocol. Ion Torrent Suite Software and Invicta Bioinformatics Team Script were used for chromosome copy number variation analysis.
Main results and the role of chance: No significant differences were observed in the mean number of metaphase II oocytes (6.8 ± 3.3; median 7; range 1-15 vs. 6.7 ± 4.3; median 6; range 1-18), fertilized oocytes (4.6 ± 2.3, median 5; range 0-10 vs. 4.9 ± 3.3, median 5; range 0-15) and top quality blastocyst (1.9 ± 1.3; median 2; range 0-6 vs. 2.3 ± 1.6; median 2; range 0-5) between FPS and LPS. The number of balanced embryos was significantly higher ($p<0.01$) in LPS compared to FPS. The mean number of balanced blastocysts in LPS was 1.2 ± 1.1; median 1; range 0-4 vs. 0.6 ± 0.5; median 0; range 0-2 in FPS. The mean rate of balanced blastocysts per MII was also significantly higher ($p<0.01$) in LPS compared to FPS (21.6% vs. 9.8% respectively).
Limitations, reasons for caution: More data are required to confirm that higher number of balanced embryos is a result of shorter recruitment time of oocytes from antral follicles.
Wider implications of the findings: The evidence of multiple follicular waves during a single menstrual cycle in women has important implications for the treatment of infertility. This strategy can maximize the number of oocytes obtained per menstrual cycle, in turn increasing the chance to obtain reproductively competent, balanced embryos in the shortest possible time.
Trial registration number: not applicable

P-663 Androgen treatment in women undergoing IVF/ICSI with poor ovarian reserve (POR): a systematic review and meta-analysis

N. Bradley[1], D. Vorster[1], L. Robinson[2], J. Ghosh[3], Y. Jeve[4], J. Chu[5]

[1]University of Birmingham, Medical School, Birmingham, United Kingdom ;
[2]Birmingham Women's and Children Hospital NHS Trust, Fertility and Menopausal Services, Birmingham, United Kingdom ;
[3]Birmingham Women's and Children Hospital NHS Trust, Academic Department of Obstetrics and Gynaecology, Birmingham, United Kingdom ;
[4]Birmingham Women's and Children Hospital NHS Trust, Reproductive Medicine and Surgery, Birmingham, United Kingdom ;
[5]Birmingham Women's and Children Hospital NHS Trust, Reproductive Medicine, Birmingham, United Kingdom

Study question: Is treatment with androgens effective for women with poor ovarian reserve undergoing IVF/ICSI treatment?
Summary answer: Current evidence suggests that Dehydroepiandrosterone (DHEA) supplementation may improve IVF/ICSI outcomes in women with POR, however, well-designed studies supporting this are lacking.
What is known already: Ovarian aging, which occurs due to a decreasing quality and quantity of oocytes, is a major factor for declining fertility. Poor ovarian reserve (POR) means women who suffer from ovarian aging have worse outcomes when undergoing assisted reproductive techniques such as IVF/ICSI due to poor response to ovarian stimulation. Exogenous steroids, such as DHEA or testosterone gel, have been used as a therapeutic intervention to improve IVF/ICSI outcomes in those with POR.
Study design, size, duration: This is a systematic review and meta-analysis of 13 RCTs encompassing 1661 patients with POF undergoing IVF/ICSI. We also conducted a study of fertility clinicians on the 'Smart Survey' platform, to gain a picture of the use of androgens (specifically DHEA) in current practice in the UK. This was open from 12/11/2019 - 27/01/2020.
Participants/materials, setting, methods: We conducted a literature search of Embase, Pub-Med, MEDLINE and Cochrane Library for articles that included the terms 'dehydroepiandrosterone', 'ovarian reserve', 'assisted reproduction techniques' 'in vitro fertilisation' and 'intracytoplasmic sperm injection'. The search was restricted to include randomised controlled trials only. Studies were excluded if they did not have live birth, clinical pregnancy rates or oocyte retrieval numbers as outcomes. We also created and distributed a retrospective cross-sectional survey to fertility settings across the UK.
Main results and the role of chance: In total 13 trials (1661 women) were included in this systematic review and meta-analysis. The meta-analysis showed that compared to the placebo, patients treated with exogenous androgens showed no improvement in live birth rate; however, there were improvements in clinical pregnancy rate and the number of oocytes retrieved during IVF/ICSI. Three trials (212 women) reported on the outcome of live birth. There was no significant difference in live birth rate between the two groups (relative risk 1.53, 95% CI 0.69-3.38, p = 0.29) with significant heterogeneity detected between these three trials (I^2=51%). Twelve studies (345 patients) reported on clinical pregnancy with the pooled analysis favouring androgen treatment (relative risk 1.41, 95% CI 1.15-1.73, p = 0.0009). Nine studies (849 patients) reported on the number of oocytes retrieved with the pooled analysis supporting the intervention (mean difference 0.80, 95% CI 0.35-1.25, p = 0.0004).

Our survey received 53 responses from a variety of UK clinicians. Of the responders, 21 (39.6%) use DHEA prior to assisted reproductive techniques (IVF/ICSI) in the treatment of patients with POR, and 17 (35.4%) would recommend the use of DHEA to a colleague.
Limitations, reasons for caution: Strict inclusion criteria were used to conduct the systematic review. As only three trials reported live birth, strong inferences cannot be made. Furthermore, we found significant statistical heterogeneity between these studies. Further interventional studies are required to assess the effectiveness of exogenous androgen in women with POR undergoing IVF/ICSI.
Wider implications of the findings: The findings of this systematic review suggest that DHEA supplementation may improve outcomes of IVF/ICSI for women with POR. However, further large, high-quality RCTs are needed to provide a clear picture of the benefits and potential risks of using DHEA as a fertility treatment.
Trial registration number: not applicable

P-664 Association between follicular fluid anti-Müllerian hormone level and oocyte quality

K. Kacem[1], B. Molka[1], M. Hamdoun[2], M. Braham[3], A. Fadhlaoui[3], F. Zhioua[3], N. Chakroun[1], O. Bahri[2]

[1]Aziza othmana hospital, cytogenetic and reproduction medecine, Tunis, Tunisia ;
[2]Aziza Othmana hospital, Biochemistry laboratory, Tunis, Tunisia ;
[3]Aziza Othmana hospital, Gynecology and obstetric, Tunis, Tunisia

Study question: What is the relationship between the anti-Müllerian hormone (AMH) concentration within
Follicles, and oocytes quality or pregnancy after ICSI and a transfer of more than one fresh embryo?
Summary answer: Among the analysed markers on the day of oocyte retrieval, AMH concentration in follicular fluid (FF)is associated with oocyte quality or pregnancy after embryo transfer.
What is known already: The serum anti-Müllerian hormone (AMH) level is a poor predictor of oocyte quality and pregnancy after IVF .However, it has been demonstrated that follicular fluid anti-Müllerian hormone of one oocyte was an excellent predictor of live birth after fresh a single embryo transfer .In fact, this embryo was matched to that oocyte.
Yet, it is still unclear whether the aptitude ofmany follicles to produce AMH reflects serum AMH concentration or their reproductive potential after embryo transfer without matching between embryos and oocytes.
Study design, size, duration: This prospective cohort study included 58 FF samples from 58 infertile women scheduled for ICSI over a 3-month period.
The FF samples were taken from at least two different follicles.
Participants/materials, setting, methods: Cycles were performed with HMG or recombinant FSH and GnRh antagonist.Concentrations of AMH in serum were measured at the start of stimulation,concentrations of AMH in follicular fluid were measured the day of oocyte pick-up.Elecsys AMH assay and electrochimiluniscence technology was used on e411 Roche cobas diagnostics to measure the two types of AMH .SPSS25 was used for the statistical analysis and comparison of averages with test for independent samples and was significant if <0,05.
Main results and the role of chance: Our patients were divided into three groups based on the follicular AMH level:

Group1: Follicular AMH <2ng/ml (29 patients)
Group2: 2ng/ml≤ Follicular AMH<3ngml (10patients)
Group3: Follicular AMH≥3ng/ml (19 patients)

The comparison of averages showed that the oocyte quality was significantly better in group 2.

In fact,the oocyte maturity rate was 72% in the second group higher than in groups 1 and 3 (59% p=0.02 and 66% p=0.048 respectively).The degenerative rate was 14,88% in group2 lower than in groups 1 and 3 (23.59% p=0.028 and 18.6% p=0.07 respectively).

The fertilisation rate was 92.38% in the second group higher than in group 1 (57.2%,p=0.001)and In group 3 (58.8%,p=0.001).

The top embryo rate was 25.48% and the pregnancy rate was 32% in the second group which is better than in group 1 (12% ;p=0.04 and 22%,p=0.058 respectively)and in group 3(15.6%,p=0.06 and 25% p=0.07 respectively).

An analysis of these FF concentrations revealed that AMH concentrations were twice as low as serum AMH in all the groups

Limitations, reasons for caution: The serum AMH concentration were not measured the day of oocyte pick up, y it was impossible to link the level of intrafollicular AMH level of a given follicule with the quality of the oocyte derived from the same follicle.

Wider implications of the findings: Group 2 was associated with a better rate of oocyte maturity,fertilisation,top embryo and pregnancy.FFAMH concentration may reflect granulosa cell proliferation during gonadotropin-stimulated follicle growth.Finally,serum AMH concentration is markedly higher than the FF AMH concentration,with no correlation between serum and FF AMH,involving ovarian follicles autonomy with regards to their secretory products.

Trial registration number: not applicable

P-665 What is the value of monitoring by ultrasonography in ovulation induction with clomiphene citrate?

J.P. De Bruin[1], S. Braam[2], N. Weiss[2], N. Lamballk[2], T. Konig[2], B.W. Mol[3], M. Van Wely[2]

[1]Jeroen Bosch Hospital, Obstetrics & Gynaecology, 's Hertogenbosch, The Netherlands ;
[2]Amsterdam UMC, Obstetrics & Gynaecology, Amsterdam, The Netherlands ;
[3]Monash University, Obstetrics & Gynaecology, Melbourne, Australia

Study question: Should women undergoing ovulation induction with clomiphene citrate (CC) be monitored with ultrasound to prevent multiple pregnancy?

Summary answer: Ultrasound monitoring with cancellation in multifollicular growth prevents multiple pregnancies but leads to lower live birth rate and higher costs.

What is known already: Ovulation induction with CC in women with normogonadotropic anovulation can lead to multifollicular growth increasing the probability of multiple pregnancy. There is no consensus on the need to monitor follicular development by transvaginal ultrasound (US).

Study design, size, duration: We performed a retrospective cohort study among 484 anovulatory infertile women treated with CC.

Participants/materials, setting, methods: Data from the first ovulatory cycle with CC in 484 women on number of dominant follicles per cycle, the number of ongoing singleton and multiple pregnancies. In order to investigate possible prediction of multifollicular growth, we performed linear regression analysis. The data were used in a decision model simulating two treatment scenarios, CC treatment with or without US monitoring and with varying outcomes such as the costs of monitoring and the incidence of multiple births.

Main results and the role of chance: In 484 women undergoing a first cycle, 393 had monofollicular growth (81.2%) resulting in 66 ongoing singleton pregnancies (16.8%). There were 73 women (15.1%) with a cycle with two dominant follicles that resulted in 22 ongoing pregnancies (30%), of which four were ongoing twin pregnancies (18.2%). There were 13 cycles (2.7%) with three dominant follicles, one of which was cancelled. These cycles led to three ongoing singleton pregnancies (23.1%).

Under the scenario that a CC cycle was not monitored by US and none of the cycles were cancelled, we estimate the ongoing pregnancy rate to be 19.2% (95% CI 16.0 to 22.9%). In this scenario the average costs for 484 basal body temperature charts (BBTs) with 6 (6.5%) twin pregnancies was estimated at € 4,272 (95% CI 3,418 to 5,127) per live birth.

Under the scenario that all cycles were monitored with US with cancellation of those with multifollicular growth, we would have cancelled 91 of the 484 cycles (cancellation rate 18.8%), thus preventing four multiple pregnancies but at the cost of 21 singleton pregnancies. This would have reduced the ongoing pregnancy rate to 13.6% and increased the average costs to € 4,721 (95% CI 3,976 to 5,466) per live birth.

Limitations, reasons for caution: Results are based on a combination of two cohort studies with limited sample size, which may have impact on the external validity of the data.

Wider implications of the findings: Our results suggest, in view of the low multiple pregnancy rate, that withholding ultrasound monitoring in CC cycles can be considered. In case ultrasound monitoring is applied, we recommend cancelling all cycles with more than one follicle to prevent multiple pregnancies and consequently accept lower pregnancy rates.

Trial registration number: -

P-666 Decreased concentrations of branched chain amino acids, tryptophan, methionine and threonine in follicular fluid of idiopathic infertile females.

I. Listorti[1], P. Bilotta[2], R. Pallisco[1], B. Tavazzi[3], G. Bilotta[1], G. Lazzarino[4], T. Capozzolo[2], A. Guarino[2], A.M. Amorini[5], M. Saab[5]

[1]Alma Res Fertility Center, Laboratory of andrology and embriology, Rome, Italy ;
[2]Alma Res Fertility Center, Obstetrics and Gynecology, Rome, Italy ;
[3]Catholic University of Rome and Fondazione Policlinico Gemelli, Institute of Biochemistry and Clinical Biochemistry, Rome, Italy ;
[4]Saint Camillus International University of Health Sciences, Biomedical and Biotechnological Sciences, Rome, Italy ;
[5]University of Catania- Italy, Department of Biomedical and Biotechnological Sciences- Division of Medical Biochemistry, Catania, Italy

Study question: To assess whether females with unexplained infertility have free amino acids concentrations in follicular fluid (FF) different from those in FF of control fertile females.

Summary answer: FF of idiopathic infertility females has lower values of valine, isoleucine, leucine, tryptophan, methionine and threonine than those in fertile female FF.

What is known already: Previous studies analyzing FF amino acid content in female with fertility problems gave conflicting results. Either increase or decrease in specific amino acids were reported in patients with endometriosis, polycystic ovarian syndrome, diminished ovarian reserve. To date, no data are available concerning free amino acid concentration in FF of females with unexplained infertility.

Study design, size, duration: Cross-sectional study in two female groups: 1) fertile female controls (n = 34); 2) idiopathic infertile females (n = 20). FF of each participant was analyzed to determine concentrations of free amino acids and amino group-containing compounds (25 compounds). Patients were enrolled and studied over a period of 16 months, from September 2018 to January 2020.

Participants/materials, setting, methods: All participants were tested for infertility and underwent oocytes retrieval; 51 of them were submitted to conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). FF of each participant was properly processed by high performance liquid chromatography (HPLC), to quantify FF concentration of free amino acids. For all patients were counted the number of cumulus-oocyte complexes (COCs), MII oocytes, zygotes and blastocysts. FF analysis was performed in blind with respect to IVF outcome measures.

Main results and the role of chance: Among the 25 free amino acids (FAA) quantified in FF, idiopathic infertile females had lower values (expressed in mmol/l) of valine (147.27 ± 41.06, $p < 0.005$), isoleucine (28.89 ± 9.62, $p < 0.001$), leucine (56.79 ± 16.12, $p < 0.01$), tryptophan (33.37 ± 8.79, $p < 0.05$), methionine (13.81 ± 7.27 $p < 0.005$) and threonine (131.11 ± 44.18, $p < 0.05$) than the corresponding concentrations found in fertile controls (valine = 189.88 ± 36.83 mmol/l; isoleucine = 39.60 ± 8.63; leucine = 67.57 ± 13.77; tryptophan = 39.32 ± 7.39; methionine 21.16 ± 5.17; threonine (159.60 ± 43.22). Due to the relatively limited number of patients, no statistical correlations were found among the concentrations of aforementioned essential amino acids in FF and IVF outcome. Although tendency to increase oocyte quality and good blastocysts rate were observed in patients with higher essential amino acids concentration. Since these defective amino acids are involved not only in protein synthesis, but also in energy-related metabolic functions and DNA or protein methylation it is possible that their defect in FF affects negatively oocyte and embryo development in unexplained infertility.

Limitations, reasons for caution: The main limitation of the study is related to the limited number of patients enrolled that did not allow reaching statistical correlations among the biochemical parameters of FF and the biological outcome measures of IVF.
Wider implications of the findings: These results showed that idiopathic infertile females have amino acid dysmetabolism signatures in FF. It would be possible supply FF with these compounds, to improve oocyte quality, embryo development and IVF outcome.
Trial registration number: not applicable

P-667 Expression alterations of enzymatic genes involved in the metabolism of branched-chain amino acids in subcutaneous adipose tissue in pregnant women with polycystic ovary syndrome.

S. Hajitarkhani[1],[2], A. Alizadeh[1], M. Shahhoseini[1], A. Moini[1]

[1]Royan Institute for Reproductive Biomedicine- ACECR- Tehran-, Department of Embryology- Reproductive Biomedicine Research Center., Tehran, Iran ;
[2]University Science and Culture Tehran- Iran, Department of Cell and Molecular Biology Science- Faculty of Basic Sciences and Advanced Technologies in Biology, Tehran, Iran

Study question: Is expression of BCAT2, BCKDHA, BCKDHB, DBT, DLD genes different in subcutaneos Adipose Tissue of pregnant women with polycystic ovary syndrome compared with control group?
Summary answer: Overexpression of BCKDHB, DBT genes and decreased expression of BCKDHA gene were detected in subcutaneos Adipose Tissue of pregnant women with polycystic ovary syndrome.
What is known already: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women at reproductive ages and is a biochemical and metabolic abnormality too. This disorder is often associated with insulin resistance. White adipose tissue is known as an active region in the metabolism of many amino acids, including branched-chain amino acids (BCAAs). Previous studies had indicated that impaired BCAAs metabolism in adipose tissue can be a prognostic factor in insulin-resistant diabetes in the future. It is necessary to investigate gene expressions related to BCAAs metabolism in adipose tissue of women with PCOS.
Study design, size, duration: In this study, we used 13 pregnant women with PCOS and 6 pregnant women without PCOS. All samples were obtained from women who had no evidence of Abortion history or women who had not used insulin treatments during the last 3 months and were Non-diabetics, Non-Smoking. No significant differences were found in age and body mass index (BMI) betwen women with PCOS and non-PCOS women. All women filled the consent letter consiously.
Participants/materials, setting, methods: Abdominal subcutaneous fat biopsy was performed during cesarean section, samples were washed, cut and snap-freezed immediately, then cDNA synthesis was done. Real-time PCR technique was used for relative gene expression of BCAT2, BCKDHA , BCKDHB , DBT , DLD. Gene expression data were analyzed based on $2^{-\Delta\Delta CT}$ to estimate the relative fold change value. It was analyzed by t-test. Differences with $P < 0.05$ were considered significant.
Main results and the role of chance: The level of mRNA expression of BCAT2 and DLD genes in PCOS samples was not significantly different from non-PCOS samples ($P > 0.05$). Level of mRNA Expression of BCKDHB and DBT genes was increased in PCOS samples compared with control samples. These increases were statistically significant ($P < 0.001$). Level of mRNA expression of BCKDHA gene was decreased in PCOS samples compared to non-PCOS samples. These decreased were statistically significant ($P = 0.001$).
Limitations, reasons for caution: For getting more information, we need to study these genes in a larger number of women with and without PCOS and this study should be performed on subcutaneous and visceral adipose tissue of non-pregnant women due to the multifactorial pathology of pregnancy.
Wider implications of the findings: Expression changes of these genes from PCOS groups can be considered as a molecular scenario for the pathophysiology and the occurrence of insulin-resistance diabetes due to impair of Branched-Chain Amino Acids metabolism in white adipose tissue.
Trial registration number: not applicable

P-668 Variation of Antimullerian Hormone (AMH) Measurements and Its Impact on Prescription and Predicted Response in Controlled Ovarian Stimulation

G. Maciel[1], G. Maffazioli[2], V. Heirich[2], C. Lopes[2], R.P. Biscolla[3], J. De Sá[3], C. Ferrer[3], J. Vieira[3], E. Baracat[2]

[1]Hospital das Clínicas HCFMUSP & Laboratorio Fleury Medicina e Saude, Obstetrics and Gynecology, Sao Paulo, Brazil ;
[2]Hospital das Clínicas HCFMUSP, Obstetrics and Gynecology, Sao Paulo, Brazil ;
[3]Laboratorio Fleury Medicina e Saude, Endocrinology, Sao Paulo, Brazil

Study question: To analyze the variation of AMH concentration in an interval <12 months and its clinical impact on the ovarian stimulation planning in assisted human reproduction.
Summary answer: Although the variation of AMH level within 12 months was low, in 19.2% of the cases it would change the ovarian stimulation protocol.
What is known already: AMH is one of the most reliable tools for the evaluation prediction of response in controlled ovarian stimulation (COS) and ovarian reserve of female reproductive capacity. Depending on AMH levels and body mass index, it is possible to predict ovarian response and to choose the best gonadotropin doses, in order to avoid cycle cancellation due to either an excessive (hyper) or poor response. However, some questions about the physiology of AMH and the stability of laboratory determination systems have been raised lately. Due to its good stability, it is considered a good indicator of ovarian response to assisted reproduction cycles**.**
Study design, size, duration: Observational retrospective study with 554 women who had measured AMH levels by the same assay in an interval of <12 months (T1 and T2) between the years of 2014 and 2018 at a large clinical analysis laboratory. Samples were divided into 3 groups according to AMH levels at T1 and the prognosis of controlled ovarian stimulation: risk of poor response (LR):<1.2 ng/mL; normal response (NR): 1.2-3.4 ng/mL; risk of ovarian hyperstimulation (rOH): >3.4ng/mL.
Participants/materials, setting, methods: Mean age of women included was 37.0±4.4 years old, FSH 11.0±16.1 IU/L, LH: 10.1±14.9 IU/L and AMH 1.60±2.44 ng/mL. Exclusion criteria were the use of hormonal contraceptive, biotin and ovulatory induction medications or follicle stimulated hormone (FSH) and/or luteinizing hormone (LH) levels < 1.0 UI/L at the time of AMH dosage. AMH was measured by platforms Elecsys™, Roche and Pico AMH™, Ansh Labs. Chi-squared statistical test was used to evaluate proportion differences between groups.
Main results and the role of chance: The mean interval between the two measurements was 6.5±3.4 months, and the mean variation of AMH was 0.05±1.86 ng/dL. At T1, 61% presented LR; 29% NR; and 10% rOH. After T2, 103 women (19%) had changed their prognosis in RHA. From these, 6.7% changed from LR to NR; 6.3% from NR to BR; 3.2% from NR to rOH; 2.6% from rOH to NR and 0.2% from LR to rOH. In a subset analysis, women were then divided into two groups according to age range: younger than 35 years old (n=161) and older than 35 years old (n=383). Mean ages for both groups were 31.9±2.7 and 39.1±3.0 years old, respectively. Mean variation of AMH levels were 0.39±2.64 ng/mL at < 35 years old group and 0.09±1.38 ng/mL at > 35 years old group. There was no difference in the proportion of prognosis change in assisted human reproduction between the two age range groups (22.9% vs 17.6%, p=0.16).
Limitations, reasons for caution: These findings are limited by lack of clinical data regarding the reason of AMH measurement, phase of the menstrual cycle and information of antral follicle count, another important predictor of ovarian response in assisted reproduction cycles. However, it raises a question about the best clinical practice when predicting ovarian response.
Wider implications of the findings: At a timeframe lower than 12 months, the mean variation of AMH was low, but enough to change the protocol and prognosis in assisted human reproduction in 19% of the cases. These data suggest that the measurement of AMH should be performed close to the beginning of the ovarian stimulation.
Trial registration number: Not applicable

P-669 which gonadotropin, recombinant or urinary,works better in aging women.

R. Hu[1], W. Yafei[2]

[1]General Hospital Of Ningxia Medical University, Reproductive Medicine Center of General Hospital Of Ningxia Medical University, Yingchuan, China ;
[2]Ningxia Medical University, Ningxia Medical University Clinical medicine, Yinchuan, China

Study question: To determine which gonadotropin, recombinant or urinary,works better in aging women from efficacy and economic perspective.
Summary answer: For aging women, superiority of HMG over rFSH and uFSH in clinical outcomes could not be concluded from this study, but noninferiority was established.
What is known already: Although potential benefits of LH activity were large quantity reported, it remains controversial whether adding LH during ovarian stimulation improves oocyte quality. A recent systematic review found that the addition of LH activity may improve ongoing pregnancy rates than FSH alone, but not live birth rates. Some papers reported that adding rLH to patients below 35 did not bring any benefit, but may be beneficial for advanced age and patients with decreased ovarian reserve. However, few reports indicated that aging women (≥35 years) have a beneficial effect on rLH supplementation during a antagonist protocol.
Study design, size, duration: This is a retrospective analysis.From January 2009 to December 2017, collecting 500 infertile women ≥35 years old undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection cycles (ICSI) received ovarian stimulation with HMG (n=199) or with uFSH (n=198) or with rFSH (n=103) in a gonadotropin-releasing hormone(GnRH) antagonist protocol.
Participants/materials, setting, methods: The primary end points were clinical pregnancy and live birth rate ,while the secondary endpoints were total dose administered to FSH, mean stimulation days, serum estradiol levels and endometrial thickness on the day of hCG administration, number of oocytes retrievaled, MII oocyte numbers, number of frozen embryos, fertilization rate, embryo cleavage rate,implantation rates, Abortion rate, cycle cancellation rate, incidence rate of OHSS and the costs per cycle, cost-effectiveness ratio of the three regimens.
Main results and the role of chance: There were no statistically significant differences in demographic data and baseline characteristics for three groups.The mean numbers of oocytes retrieved was lower in HMG group compared with uFSH and rFSH groups (4.6±2.9 vs. 6.2±3.8 vs. 7.1±4.7,P=0.000), while the clinical pregnancy per started cycle (23.6 vs. 27.3 vs. 31.1%,P=0.367), implantation (16.1 vs. 20.8 vs. 22.1%) and live birth rate per started cycles (13.6 vs. 20.2 vs. 20.4%) were comparable. Data were further analyzed performing separate comparisons in subpopulations with different ranges of age, i.e.35-37, 38-40, and >40. Major differences between the three regimens were observed in women with age>40. In this subpopulation, not only the implantation rate was higher in the uFSH group compared with HMG and rFSH groups (15.4 vs. 4.4 vs. 13.0%, $P < 0.05$), but also live birth rate was significantly higher (12.9 vs. 1.3 vs. 6.7%, $P < 0.05$). The cost-effectiveness ratio in the HMG group was $564.6 and $2,170.5 in the uFSH group and $17,561.3 in the rFSH group.
Limitations, reasons for caution: However, considering its retrospective study, there is a certain limit to the evidence. It is strongly suggests the necessity to carry out large RCTs for patients with advanced age or DOR to guide clinical applications.
Wider implications of the findings: In the era of individualized treatment, this study provides the most effective and economical treatment options for aging women undergoing IVF and ICSI treatment.
Trial registration number: not applicable

P-670 Oophorectomy in pre-menopausal women, long-term risk of breast cancer and the modifying effects of menopausal hormone therapy: A prospective cohort study.

T. Koch[1], J. Therming Jørgensen[2], L. Priskorn[1], A. Juul[1], C. Dehlendorff[3], A.K. Dunn-Henriksen[3], J. Christensen[3], E. Bräuner[1], Z. Andersen[2], M. Hickey[4]

[1]Rigshospitalet, Growth and Reproduction, Copenhagen, Denmark ;
[2]University of Copenhagen, Section of Environmental Health- Department of Public Health, Copenhagen, Denmark ;
[3]Danish Cancer Society Research Center, Statistics and Pharmacoepidemiology, Copenhagen, Denmark ;
[4]University of Melbourne, Obstetrics and Gynaecology, Melbourne, Australia

Study question: Does pre-menopausal oophorectomy (surgical menopause) change the long-term risk of breast cancer and how does menopausal hormone therapy modify this risk?
Summary answer: Statistical analyses are ongoing, results will be ready prior to the meeting in July 2020
What is known already: Existing evidence suggests that pre-menopausal oophorectomy reduces the risk of breast cancer in the general population and in women at high inherited risk of breast cancer. However, the effect of menopausal hormone therapy (MHT) on breast cancer after oophorectomy is poorly understood. This is of clinical importance because MHT is widely recommended after surgical menopause in women without contraindications. However, existing evidence is limited by self-report of oophorectomy and use of MHT potentially leading to misclassification bias.
Study design, size, duration: Prospective register-based cohort study of 28,731 participants in the Danish Nurse Cohort with detailed lifestyle information. Information about oophorectomy (unilateral or bilateral) and hysterectomy was recorded in national patient registers, use of MHT was recorded in national prescription registries and incidence of breast cancer following oophorectomy was identified from the Danish Cancer Registry using a unique identification code.
Participants/materials, setting, methods: Cox regression analyses with age as underlying timeline is used to assess the effects of pre-menopausal oophorectomy (stratified according to unilateral and bilateral) on the subsequent risk of breast cancer. Analyses are adjusted for potential confounders. Further, the modifying effects of MHT, hysterectomy (registry-based date of procedure), BMI and work patterns including shiftwork are evaluated.
Main results and the role of chance: Of the 28,731 nurses we excluded 632 with a breast cancer diagnosis before baseline, 645 with a diagnosis of other cancer (not non-melanoma skin cancer) before baseline, 505 with mastectomy before baseline and 3 with an inactive status in CPR before baseline leaving nurses 27,422 eligible for follow-up. During follow-up, we identified 2,165 cases of breast cancer.

Breast cancer cases had a slightly higher median baseline age, were more likely to have had a bilateral oophorectomy and use of MHT, but were similar in regard to unilateral oophorectomy, hysterectomy, use of oral contraceptive, parity, age at menarche, physical activity and working conditions. Further, cases were older when they had their first child and were more likely to have an unhealthy lifestyle (higher BMI, current smokers and heavy drinkers) compared to nurses without breast cancer. Regression analyses are being executed and results will be ready before ESHRE2020.
Limitations, reasons for caution: Gene mutation status is not available so the indication of risk-reducing oophorectomy cannot be determined. Genetic variants known to affect breast cancer risk such as BRCA1/BRCA2 are not known. Secondly, confounder variables (for example smoking, alcohol, BMI, etc.) are based on baseline questionnaire data and may have changed during follow-up.
Wider implications of the findings: Bilateral salpingo-oophorectomy is the only evidence-based intervention to reduce morbidity and mortality from ovarian cancer. However, the optimum timing of risk-reducing oophorectomy and the effects of MHT usage are uncertain. This study will provide new information to inform clinical practice in this area and may translate into improved health outcomes.
Trial registration number: Not applicable, this is a register-based study.

P-671 Association of luteinizing hormone/choriogonadotropin receptor G935A and Ins18LQ gene polymorphisms with polycystic ovary syndrome in Indonesian women

H.P. Lubis[1], M.F.G. Siregar[1], B. Halim[1], I. Adenin[1]

[1]Faculty of Medicine- Universitas Sumatera Utara, Obstetrics and Gynecology, Medan, Indonesia

Study question: Is there an association between luteinizing hormone/choriogonadotropin receptor (LHCGR) G935A and ins18LQ gene polymorphisms and polycystic ovary syndrome (PCOS) in Indonesian women?
Summary answer: Luteinizing hormone/choriogonadotropin receptor (LHCGR) G935A and ins18LQ gene polymorphisms were not associated with PCOS in Indonesian women.
What is known already: Polycystic ovary syndrome (PCOS) is one of the most common endocrine-metabolic disorders and a complex genetic disorder. Study on genes susceptible to PCOS has focused mainly on gene polymorphisms that encode sex hormones and regulatory proteins such as follicle-stimulating hormone ß (FSHB), follicle-stimulating hormone receptor (FSHR), Luteinizing Hormone Chorionic Gonadotropin Receptor (LHCGR), estrogen receptor alpha receptor (ESR1), and estrogen receptor ß (ESR2). Variants of genetic markers have implications for the predisposition of PCOS, however, there are no variants that are directly and repeatedly linked to PCOS.

Study design, size, duration: A case-control study was performed in 50 women with PCOS as the case group and 50 healthy women with none of the pathological characteristics as the control group from May 2019 until October 2019 in Halim Fertility Center, Division of Reproductive Endocrinology and Infertility, Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
Participants/materials, setting, methods: All participants were women of reproductive age. Polymorphisms of LHCGR G935A and ins18LQ genes were genotyped in all subjects using *polymerase chain reaction-restriction fragment length polymorphism* (PCR-RFLP). The allelic frequencies of each case group were compared with the controls.
Main results and the role of chance: From this study, we found that there was no significant association between LHCGR G935A and ins18LQ gene polymorphisms with PCOS. There were significant differences in the characteristics of Body Mass Index (BMI), FSH level, LH level, and LH / FSH ratio between the PCOS and control groups ($p<0.05$). In the LHCGR G935A gene polymorphisms, the proportion of homozygote mutant AA genotypes was higher in the PCOS group (6%) compared to the control group but there was no significant difference between them ($p= 0.391$). The frequency of allele A was higher in the PCOS group compared to the control group. Then, in the LHCGR ins18LQ gene polymorphisms, the proportion of heterozygote variant nonins/ins was higher in the PCOS group (12%) than the control group (4%) but there was no significant difference between the polymorphisms of the nonins-ins and nonins-nonins variants between the PCOS and control groups ($p=0.269$). The frequency of ins allele was higher in the PCOS than the control group.
Limitations, reasons for caution: The sample size of this study was relatively small, which therefore limited the statistical power of the analysis to a certain extent.
Wider implications of the findings: This is the first report studying the association of LHCGR G935A and ins18LQ gene polymorphisms with PCOS in Indonesia. The increased frequency of homozygote mutant genotypes of LHCGR gene in PCOS group suggests that these subjects have an elevated risk of developing the syndrome, although the association is not significant.
Trial registration number: not applicable

P-672 Long-term effects of premenopausal oophorectomy on mental health and modifying effects of Menopausal Hormone Therapy. A prospective Cohort Study.

E. Bräuner[1], T. Koch[1], L. Priskorn[1], A. Juul[1], C. Dehlendorff[2], J. Christensen[2], A.K. Dunn-Henriksen[2], J. Therming Jørgensen[3], Z. Andersen[3], M. Hickey[4]

[1]Rigshospitalet, Growth and Reproduction, Copenhagen, Denmark ;
[2]Danish Cancer Society Research Center, Statistics and Pharmacoepidemiology, Copenhagen, Denmark ;
[3]University of Copenhagen, Section of Environmental Health- Department of Public Health, Copenhagen, Denmark ;
[4]University of Melbourne, Obstetrics and Gynaecology, Melbourne, Australia

Study question: Does premenopausal oophorectomy affect the long-term risk of depression, dementia, Parkinsonism and Alzheimer's Disease? How does Menopausal Hormone Therapy (MHT) modify this risk?
Summary answer: Preliminary analysis from this large prospective cohort study indicates that premenopausal oophorectomy increases both depression and dementia. All analysis will be complete for ESHRE2020.
What is known already: Premenopausal oophorectomy (surgical removal of one/both ovaries) is performed in around 30% of hysterectomies for benign gynecological conditions and is the only intervention shown to reduce morbidity and mortality from ovarian cancer in women at high genetic risk. Premenopausal bilateral oophorectomy leads to an immediate reduction in circulating sex steroids. Preclinical and clinical evidence indicates that ovarian sex steroids particularly estrogen, have neuroprotective action and that oophorectomy may increase depression, Parkinson's Disease and dementia. However, existing studies are limited, the evidence is conflicting, the modifying effects of MHT are uncertain.
Study design, size, duration: The nationwide Danish Nurses Cohort (est. 1993, n=28,731 women) will be used to prospectively determine the long-term effects of premenopausal oophorectomy on dementia, Parkinsonism, Alzheimer's Disease and depression (symptoms/major disorder) risk and the modifying effects MHT on this. Extensive baseline data including socio-economic/working conditions, weight, height, lifestyle and reproductive history/health have been collected. Information on oophorectomy, health outcomes and MHT are extracted from national patient and prescription registers and data access is approved.
Participants/materials, setting, methods: Time-varying cox regression models, with age as underlying timeline are used to assess the risk of each outcome separately. All nurses (exposed and unexposed, matched by age on the index date of oophorectomy) are followed up for the date of outcomes of interest (separate analyses), death, disappearance and emigration and censored on that date or end of follow-up (31st December 2018), whichever comes first. Modifying effects of MHT use, BMI and hysterectomy will be measured.
Main results and the role of chance: Participants with cancer (apart from non-melanoma skin cancer) and those with missing baseline information have been excluded, leaving 22,882 female nurses for the analyses. Of these 22,882 women, 1,084 (4.7%) nurses underwent premenopausal oophorectomy (unilateral/bilateral: 685/399) and 2,262 (9.9%) underwent post-menopausal oophorectomy (unilateral/bilateral: 685/399) defined as age > 51 years. During a mean follow-up of 18.6 years, 8,077 nurses developed depression (depressive symptoms or major disorder), 1,534 developed dementia, 1,013 Parkinson's Disease and 1,157 Alzheimer's Disease. In general, cases had a slightly higher median age at baseline, were more likely to have had an oophorectomy, but were similar in regard to hysterectomy, use of MHT, use of oral contraceptive, parity, age at menarche, physical activity and working conditions. Further, cases were more likely to have an unhealthy lifestyle (higher BMI, current smokers and heavy drinkers) compared referent nurses. We will report hazard ratios (HRs) reflecting the risk of these outcomes stratified according to unilateral and bilateral oophorectomy and further stratified according to reproductive stage at oophorectomy (pre or postmenopausal). Cox regression analyses are in progress, but preliminary results suggest significantly increased risks for depression and dementia and borderline effects for Parkinsonism and Alzheimer's Disease.
Limitations, reasons for caution: Gene mutation status is not available so the indication of risk-reducing oophorectomy cannot be determined. Also, genetic variants known to affect dementia risk such as genetic information APOE or ESR1 are not known. Secondly, confounder variables are based on baseline questionnaire data and may have changed during follow-up.
Wider implications of the findings: The global health burden of mental health disease in women is enormous and understanding the modifiable factors contributing to these is of international importance. This will be the first prospective cohort study investigating these risks after premenopausal oophorectomy, generating new evidence to inform clinical practice and improve health outcomes.
Trial registration number: Not applicable, this is a register-based study.

P-673 Physiological temperature in human follicles during oocyte growth is around 35°C: does lowering temperature to 35°C affects maturation rate and quality of human oocytes?

D. Nikiforov[1], J. Cadenas[1], S.E. Pors[1], L.A.Z. Jara[1], W. Yu[1], S. Gry Kristensen[1], C. Yding Andersen[1]

[1]Laboratory of Reproductive Biology, The Juliane Marie Centre for Women-Children and Reproduction- Copenhagen University Hospital, Copenhagen, Denmark

Study question: Does the physiological temperature of 35°C, observed within large antral human follicles *in vivo*, affect maturation rate of human oocytes undergoing *in vitro* maturation (IVM)?
Summary answer: Immature human oocytes cultured at 35°C demonstrated a similar maturation rate and morphological quality as immature oocytes matured at 37°C.
What is known already: Studies on several mammalian species, including humans, revealed that the temperature inside large antral follicles is lower compared to the surrounding stroma. Within the human ovary, this difference is, on average, 2.3°C. Therefore, growing oocytes are under physiological conditions exposed to temperatures below 37°C. No studies have evaluated the maturation capacity of human oocytes at different temperatures. This study aimed to compare nuclear maturation (i.e., MII-transition of GV-oocytes) of human oocytes after IVM at 35°C (*in vivo* temperature inside preovulatory follicle) versus 37°C (deep body temperature in humans). Correlation between oocytes' quality, diameter and maturation rate were also analyzed.

Study design, size, duration: In a prospective cohort study, patients undergoing ovarian tissue cryopreservation (OTC) were recruited. After isolation and cryopreservation of ovarian cortex, the remaining medulla tissue was examined for the presence of immature oocytes, released from small antral follicles during dissection. Oocytes were collected and matured *in vitro* for 44-48 hours following standard IVM procedures. The oocytes were randomly allocated to incubation at 35 °C or 37°C.

Participants/materials, setting, methods: Twenty-five patients, aged 19-38 years, were included. The MII rate was compared between oocytes cultured at 35°C vs 37°C. Temperature monitoring was ensured by wireless pre-equilibrated data logging devices controlling the temperature inside both incubators. After IVM, oocyte nuclear maturation, diameter, and morphology were evaluated.

Main results and the role of chance: On average, 25 immature oocytes were collected from each patient (range 6-53, N=637). No significant difference was found in the overall maturation rate between oocytes incubated at 35°C and 37°C (37% vs 33%, respectively; p=0.25). When analyzing the maturation rate in a subgroup of oocytes found in large cumulus-oocyte complexes (COCs) (> 15-19 layers of cumulus cells), a significantly higher rate was observed in oocytes incubated at 35°C as compared to 37°C (MII rate 52% vs 42%, respectively; p=0.03). Furthermore, oocyte diameter was found to be significantly associated with maturation rate, regardless of cumulus size (large COCs, small COCs or naked oocytes) or temperature: oocytes with larger diameter had better chances for maturation (p<0.0001). Oocyte quality evaluation based on morphology, which comprised a strict assessment of cytoplasm, perivitelline space and zona pellucida, demonstrated no differences after maturation at the two temperatures (p=0.25) and was not related to oocyte diameter (p=0.09).

Limitations, reasons for caution: A limitation of the study is the lack of data regarding the developmental potential of MII oocytes after IVM. Additional data regarding fertilization and blastulation rates of oocytes may provide insight into the impact of temperature during IVM on developing embryos.

Wider implications of the findings: The study demonstrated that the temperature range for IVM of human oocytes is surprisingly wide. *In vitro* maturation of oocytes collected from small antral follicles had sufficient MII rate at both 35°C and 37°C. The use of IVM in connection with OTC should be considered clinically.

Trial registration number: not applicable

P-674 Serum progesterone measurement in frozen embryo transfers following hormone replacement therapy: a systematic review and meta-analysis

P. Melo[1], Y. Chung[1], O. Pickering[1], M.J. Price[1], S. Fishel[2], M. Khairy[2], P. Lowe[3], M. Rajkhowa[2], A. Tozer[4], M. Wilcox[4], I.D. Gallos[1], A. Coomarasamy[1]

[1]Tommy's National Centre for Miscarriage Research, Birmingham Women's Hospital, Birmingham, United Kingdom ;
[2]CARE Fertility, ProFET, Birmingham, United Kingdom ;
[3]CARE Fertility, ProFET, Manchester, United Kingdom ;
[4]CARE Fertility, ProFET, London, United Kingdom

Study question: How do serum progesterone levels in frozen embryo transfers following hormone replacement therapy (HRT-FET) correlate with pregnancy outcomes?

Summary answer: High serum progesterone is associated with an increased chance of clinical pregnancy and reduced risk of miscarriage in HRT-FET, although high-quality prospective studies are required.

What is known already: Endometrial receptivity is one of the principal factors influencing early pregnancy success in assisted reproductive treatment (ART). Progesterone is essential to ensure optimal endometrial receptivity, and in its absence, pregnancy inevitably fails. In women undergoing frozen embryo cycles, exogenous progesterone is administered to ensure synchrony between the endometrium and the developing embryo. There is conflicting evidence on the association between serum progesterone and pregnancy outcomes in HRT-FET, and no consensus exists among clinicians as to whether serum progesterone testing should be used routinely for predicting pregnancy outcomes in HRT-FET cycles.

Study design, size, duration: A systematic review and meta-analysis was performed. Electronic searches of the Cochrane Gynaecology and Fertility Specialised Register of Controlled Trials, CENTRAL, MEDLINE, EMBASE, PsycINFO, CINAHL and ClinicalTrials.gov were conducted to December 2019 to identify relevant cohort, case-control and randomised controlled studies where serum progesterone was measured around the time of HRT-FET. The key search terms were the following: [MeSH/Emtree] progesterone AND (in vitro fertilization OR intracytoplasmic sperm injection, frozen embryo, embryo transfer, assisted reproduction techniques).

Participants/materials, setting, methods: We included studies where serum progesterone was measured during HRT-FET cycles (any time from the onset of progesterone supplementation to the day of pregnancy test) and correlated with pregnancy outcomes. The primary outcome was ongoing pregnancy/live birth rate (OPR/LBR); secondary outcomes included clinical pregnancy rate (CPR) and miscarriage rate (MR). Two authors screened studies and extracted data independently. Risk ratios (RR) were calculated with 95% confidence intervals (CI). Heterogeneity was examined with the I^2 statistic.

Main results and the role of chance: Six studies were included in the meta-analysis, involving 4,369 women. Seven additional studies are awaiting classification, and two are ongoing.

The final meta-analyses consisted of data from cohort studies; five were published in peer-reviewed journals and one as a conference abstract. All studies were retrospective and reported on pregnancy outcomes according to serum progesterone concentrations. The progesterone cut-off values ranged between 32 and 50 nmol/L.

On comparing high *versus* low serum progesterone, the evidence was uncertain on the effect of high serum progesterone upon OPR/LBR compared to low serum progesterone (three studies, 3,834 women, RR 1.51, 95% CI 1.00 to 2.27). Nevertheless, high serum progesterone was found to result in more clinical pregnancies compared with low serum progesterone (four studies, 4,121 women, RR 1.39, 95% CI 1.05 to 1.84). Furthermore, high serum progesterone was linked to a reduction in miscarriage rate compared to low progesterone (four studies, 721 women, RR 0.45, 95% CI 0.29 to 0.69). None of the studies reported on any adverse events attributable to exogenous progesterone supplementation.

The included studies scored well on the Newcastle-Ottawa quality assessment scale.

Limitations, reasons for caution: The high degree of clinical heterogeneity owing to various administration routes, timings of testing and serum progesterone thresholds, as well as the retrospective nature of all studies, were the main limitations of this review. This precludes the generalisation of findings and limits the clinical applicability of this test to date.

Wider implications of the findings: Although higher serum progesterone was linked to an increased chance of clinical pregnancy and reduced risk of miscarriage, the optimal progesterone level required for HRT-FET treatment success remains uncertain. High-quality, adequately powered prospective studies are required to further investigate the diagnostic and prognostic value of serum progesterone in HRT-FET cycles.

Trial registration number: Not applicable

P-675 Serum progesterone measurement and optimization reduce pregnancy loss in frozen-thawed embryo transfer cycles with hormone replacement therapy

D. Tarasconi[1], M. Aura[1], M. Arque[1], M. Julia[1], M.Á. Checa[1], J.J. Espinós[1]

[1]FERTTY Centro de Reproducción Asistida, Medicina Reproductiva, Barcelona, Spain

Study question: Does progesterone (P) optimization by measuring and correcting serum levels when appropriate reduce pregnancy loss following IVF-ET?

Summary answer: Serum progesterone determination and its supplementation by adding subcutaneous injections when P<10,0ng/ml reduced miscarriage rates among patients undergoing ET using their own oocytes

What is known already: Progesterone has been consistently associated with pregnancy achievement and development. A significant number of biochemical pregnancies and miscarriages following In Vitro Fertilization - Embryo Transfers (IVF-ET) might be due to progesterone deficiency. Previous studies suggest that serum progesterone values lower than 9,2 to 10,64 ng/ml are detrimental to achieve optimal reproductive results in artificial replaced cycles for frozen-thawed embryo transfer. However, the best approach in daily practice combining both effiency and patient convenience is yet to be established

Study design, size, duration: Cohort study from March 2018 to December 2019 (22 months) in a private infertility centre. We included 153 IVF-ET cycles of frozen-thawed embryo transfers, with a conventional hormone replacement therapy (HRT) for endometrial preparation

Participants/materials, setting, methods: Patients with HRT in order to transfer frozen embryos. All women were treated with a sequencial combination of oral estradiol valerate (EV)(6 mg/day) for 11-15 days followed of EV plus vaginal micronized progesterone (P)(200mg/8h). In group study, at 4th day of P administration we determined serum P. With levels lowers than 10ng/dl we added 25 mg of daily subcutaneous progesterone. Conversely, patients from the control group were routinely treated. Primary outcome was ongoing pregnancy rate

Main results and the role of chance: We determined serum P values in 89 patients (study group). P levels < 10.0 ng/dl were found in 26 (29.3%), with a median value of 8.8 ng/dl (7.4–9.2). Average P levels in the rest of the group was 13.39 ng/dl (11.5–16.2). Both groups were comparable to all the parameters studied, including ongoing pregnancy rate (*P*=1.0) and pregnancy loss (*P*=0.3). Age, BMI and embryo quality were also equivalent between the study and control (N= 64) groups. The miscarriage rate was lower in the study group (10.8% versus 30%; *P* = 0.03, 95% CI) without diferences in ongoing pregnancy rates (46.1% versus 44.1%; *P* = 0.86).

Limitations, reasons for caution: As we want to combine both efficiency and patient convenience we do not perform a ¨second-look¨ in progesterone levels after supplementation to verify if we achieved better serum progesterone concentration in these patients, so this missing data could be a limitation of the study.

Wider implications of the findings: The present study suggests that with only one additional blood determination of serum progesterone, on the day prior the embryo transfer, may identify patients who will benefit of additional subcutaneous progesterone in the luteal phase support in cycles of ET with HRT using their own oocytes, reducing the miscarriage rate.

Trial registration number: not applicable

P-676 Empty follicle syndrome(EFS) in PCOS patients after GnRH agonist trigger at a tertiary level infertility centre in India: A prospective cohort study

Nayar K.D.(1)*[kdnayar@usa.net], S. Gupta[1], M. Singh[1], R. Bhattacharya[1], G. Kant[1], R. Gahlot[1], N. Sharma[1], D. Nayar[1]

[1]Akanksha IVF Centre, Reproductive Medicine, New Delhi, India

Study question: To analyze the incidence and underlying physiology of EFS following GnRH agonist trigger in PCOS patients at a tertiary level infertility centre in India.

Summary answer: EFS following GnRH agonist trigger in PCOS is a rare event. The False EFS group can have favorable outcomes following rescue trigger.

What is known already: EFS is failure to retrieve oocytes after ovarian stimulation, despite normal follicular development, incidence being 0.045%–7% . EFS is diagnosed retrospectively, since it cannot be predicted by USG or hormonal levels. GnRH agonist trigger acts on the pituitary and causes gonadotropin release leading to LH surge lasting for 24-36hours in comparison to hCG trigger where it lasts for 8-9days. False EFS is mainly due to human error in timing, administration of trigger or manufacturing and cold chain problem. For genuine EFS, receptor polymorphisms, inability of the pituitary to release gonadotropins and dysfunctional folliculogenesis due to PCOS are implicated.

Study design, size, duration: A prospective cohort study including 225 patients diagnosed with PCOS according to Rotterdam`s criteria was carried out between January 1, 2017 through 31 December 2019.

Participants/materials, setting, methods: All patients underwent Controlled ovarian hyperstimulation using fixed GnRH antagonist protocol and GnRH agonist trigger. If no oocytes were retrieved from one ovary, serum progesterone levels were done to classify as genuineEFS (S. progesterone levels >3.5ng/ml) or false EFS(S. progesterone < 3.5ng/ml). In cases of False EFS, rescue hCG trigger was given and ovum pick up scheduled 35 hours after the trigger. Freeze all strategy was employed and embryo transfer done in a subsequent cycle.

Main results and the role of chance: Incidence of EFS in PCOS patients following GnRH agonist trigger was 3.11%(7/225). The age, BMI , parity, cause and duration of infertility were similar in EFS and non EFS group. There was no significant difference in AMH and AFC levels between the two groups. However, significantly higher doses of gonadotropins(2500±743 vs. 1850±690; p=0.02) and prologend duration of stimulation(11.6±1.79 vs. 9.5±1.2; p=0.001) was noted in the EFS group. Out of 7 cases, False EFS was identified in 5 cases(71.43%) and 2 cases (28.57%) were attributed to Genuine EFS, wherein no cause was identified. Out of 5 False EFS cases, eggs were retrieved in 4 patients following rescue hcg trigger and 2 patients achieved a clinical pregnancy(40%). For Genuine EFS cases, GnRH antagonist protocol with Dual trigger was planned in the subsequent cycle. Eggs were retreived in one patient, however genuine EFS recurred in the second patient.

Limitations, reasons for caution: Although it is a prospective study, it has limitation of small sample size.

Wider implications of the findings: Our experience at a tertiary infertility care centre in India suggests that EFS is a rare occurrence in PCOS patients following GnRH agonist trigger. False EFS can have favourable outcomes following the rescue trigger and Genuine EFS is most likely attributed to intrinsic ovarian dysfunction.

Trial registration number: MCDH/2019/17

P-677 The selection of ovulation induction protocols in patients with PCO syndrome undergoing IVF procedures

J. Bila[1], S. Spremovic Radjenovic[1], A. Gudovic[1], G. Srejic[1]

[1]Clinical Centre of Serbia, Clinic for Gynecology and Obstetrics, Belgrade, Serbia

Study question: To examine the significance of the ovulation induction protocols selection in patients with PCOS in successful IVF cycles.

Summary answer: Short protocol with antagonists has proved to be the most efficient for conception. Positive predictors of conception were younger age and lower serum progesterone levels.

What is known already: Polycystic Ovary Syndrome (PCOS) is a complex endocrine syndrome that affects about 6% of women during their reproduction period. PCOS is one of the leading factors of anovulation-induced infertility and the most frequent endocrine cause for the IVF procedures. In PCOS patients, stimulation of ovulation results in maturation of more follicles and therefore increases the chances of getting the quality and healthy egg cells. However, at the same time the risk of severe OHSS might be increased.

Study design, size, duration: In this retrospective study we have included 332 patients with PCOS in the period between 2014-2018 at the Clinic for Gynecology and Obstetrics Clinical Centre of Serbia. Study inclusion criteria were: age less than 40, PCOS diagnosed prior to IVF procedures and absence of other infertility-associated factors. Study exclusion criteria were: age over 40, the presence of other infertility-associated conditions like other endocrine disorders, endometriosis or tubal factor infertility and male infertility.

Participants/materials, setting, methods: In this study were included patients who underwent both short and long stimulation protocols. Patients undergoing long protocol (A group) were on the GnRH agonist-protocol. Patients undergoing short protocols were divided in two groups – the first (B1) group using GnRH agonist-protocol and the second group (B2) group using GnRH antagonist-protocol. The protocol selection was based on the patient's age, endocrine status (FSH, LH, E2, Pg and AMH serum levels) and antral follicles count (AFC).

Main results and the role of chance: An average patient's age was 35.3 ± 3.6, average BMI was 22.6 ± 2.8. Out of 332 women included in the study, 274 (82.5%) were in the short protocol groups and 58 (17.5%) were in the long protocol group.

Short protocol with antagonist proved to be the most efficient one for the favorable outcome (69.4%), compared to short protocol with agonist (6.5%) and long protocol with agonist (24.1%).

131 patients have conceived (39.5%), regardless on used protocol. Out of 131 conceptions, 62.9% of pregnancies resulted in live births and 37.1% resulted in pregnancy termination.

Statistical analysis of conception rates between ovulation-induction protocols has shown that age (p<0.001), BMI (p=0.03) and progesterone levels (p=0.04) were statistically significantly different in favor of short protocol. Mean age, BMI and progesterone level median in patients who successfully conceived were 34.4±3.5, 22±2.6 and 0.89 ng/mL, respectively. Mean age, BMI and progesterone level median in patients who didn't conceive were 36±3.6, 22.8±2.7 and 1ng/mL, respectively.

These variables (age, BMI and progesterone level) were included in Multiple Binary Regression Model with successful conception described as a dependent variable. The Multivariate Logistic Regression analysis confirmed that age (p<0.001) and progesterone levels (p=0.027) were predictors for the successful outcome.

Limitations, reasons for caution: Despite the satisfactory number of patients the limitations were the retrospective study design, as well as the common use of short protocol with antagonists as the recommended treatment for patients with PCOS in IVF.

Wider implications of the findings: Further prospective studies would be recommended, especially in the context of determining the endocrine milieu of obtained follicles using the short protocol in patients with PCOS, as well as assessing the quality of oocytes and embryos, and factors that may affect implantation.

Trial registration number: not applicable

P-678 Is there a critical LH level for hCG trigger after the detection of LH surge in modified natural frozen thawed blastocyst transfer cycles? Clinical outcomes.

Y. Sahin[1], S. Kahraman[1], U. Gokturk[1], B. Yuksel[1], F. Gokdagli Sagir[1], G. Ozer[1]

[1]Istanbul Sisli Memorial Hospital, Assisted Reprodutive Technologies and Reproductive Genetics Center, Istanbul, Turkey

Study question: After the detection of LH surge, are different LH levels on hCG day or the day before hCG trigger significant in modified natural cycles?

Summary answer: Once LH surge has been detected, LH levels on hCG day or the day before hCG trigger does not significantly affect clinical outcomes.

What is known already: Opinions are divided on the appropriate timing of hCG with regard to LH hormone levels in modified natural cycles for frozen thawed blastocyst transfer (mNC FET). Two studies report a deleterious effect of hCG triggering on implantation and pregnancy rates in spontaneous LH rise patients (Fatemi et al. 2010, Litwicka et al. 2017), proposing that high LH levels at the time of hCG are associated with extremely low implantation rates. Results of studies focusing on LH levels at the time of hCG trigger have been variable and optimal LH level on hCG trigger day in mNC FET is controversial.

Study design, size, duration: This retrospective study was based on 1163 mNC-FET cycles initiated between January 2015 and September 2019 in Istanbul Memorial Hospital. Patients were divided into 4 groups according to serum LH levels on hCG day or the day before hCG. Group A: LH the day before hCG (n=282), Group B: LH >16-25 (n=245), Group C: LH 26-40 (n=253) and Group D, LH >40 (n=383). LH surge was defined as ≥15mI/mL.

Participants/materials, setting, methods: 1163 cycles were evaluated. Inclusion criteria were: age <43 years, body-mass index <35 kg/m2, repeated pregnancy losses cases (<3), implantation failure (<3 cycles), endometrial thickness ≥ 8 mm on HCG day. Patients were monitored to detect an LH surge and the presence of a dominant follicle, using serum assays of LH and e2 and transvaginal ultrasound scans. Patient and cycle characteristics, LH and e2 levels and clinical outcomes were analysed.

Main results and the role of chance: There was no significant difference between LH level groups in relation to patient characteristics such as mean age, body-mass index, Anti Mullerian Hormone, cycle length and cycle characteristics such as embryo grade, mean numbers of COC, MII oocyte, fertilized oocytes and the mean follicle size, e2 level and endometrial thickness on HCG day. Subgroup analyses of mNC-FET groups showed that there was no significant difference between groups in terms of implantation, clinical and ongoing pregnancy rates.

Once LH surge has been detected, triggering ovulation by hCG in patients with different LH levels undergoing mNC-FET does not have a detrimental effect on cycle outcome.

Limitations, reasons for caution: The study was a retrospective analysis.

Wider implications of the findings: mNC-FET reduces the number of hospital visits, strict ultrasonographic evaluations and blood tests required. Once LH surge has been detected, the timing of hCG can be confidently determined and patients advised accordingly.

Trial registration number: none

P-679 Comparison of patterns of endometrial receptivity biomarkers between IVF treatments performed with different gonadotrophin regimens

D. Bortolotti[1], G. Orlando[2], E. Gravotta[2], V. Gentili[1], M.G. Bafaro[3], B. Roset[3], G. Coticchio[3], A. Borini[3], R. Rizzo[4]

[1]University of Ferrara, Department of Chemical and Pharmaceutical Sciences, Ferrara, Italy ;
[2]Merck Italy, Medical Affairs Department, Rome, Italy ;
[3]9.baby family and fertility center, Reproductive Medicine IVF clinical director unit, Bologna, Italy ;
[4]University Of Ferrara, Department of Chemical and Pharmaceuticals Sciences, Ferrara, Italy

Study question: Do alternative regimens of exogenous gonadotrophins differently affect the expression of biomarkers of endometrial receptivity in IVF treatments?

Summary answer: We identified a significant difference between ART stimulation protocols (FSH+LH; FSH+HCG) in relation to the expression of selected biomarkers of endometrial receptivity.

What is known already: A highly coordinated complex sequence of biochemical and cellular events is the basis the so-called "window of uterine receptivity", during the mid-luteal phase of each menstrual cycle. However, to date, there are extremely few studies reporting on implantation biomarkers expressed in endometrial tissue following the use of different ART stimulation protocols (FSH+LH; FSH+HCG). Novel finding in this context might contribute to improve assisted reproductive technology procedures, whose rates of implantation failure remain a major problem.

Study design, size, duration: Ten endometrial biopsies and uterine flushing samples were collected during freeze-all ART treatment protocols (N=5 FSH+LH; N= 5 FSH+HCG). The endometrial receptivity status was assessed detecting molecules and immune cells implicated in immune-regulation during embryo implantation and pregnancy.

Participants/materials, setting, methods: Endometrial cells were analyzed for L-selectin ligand and Mucin-1 expression. Uterine flushing samples were analyzed for the levels of soluble molecules ((LIF (leukemia inhibitory factor); HB-EGF (heparin-binding –epidermal growth factor); Glycodelin-A; HLA (Human leukocyte antigen)-G and HLA-E; MCP-1, IP-10, IL-1β and TNF-α. Endometrial immune cells (NK cells, T cells, monocytes) were evaluated for their immune-phenotype.

Main results and the role of chance: We observed induction in Mucin-1 expression in FSH+HCG treated women. FSH+LH treated women presented a significant increase in L-selectin expression. Women treated with FSH + LH presented the highest levels of all the analyzed soluble molecules in uterine flushing samples. Interestingly, a woman with an ongoing pregnancy presented the highest levels of sHLA-G, sHLA-E, IP10, MCP1, LIF, HBGF, Glycodeln-A, IL-1beta and TNF-alpha in uterine flushing sample. The analysis of cell content in endometrial biopsies showed a lower amount of endometrial NK cells in endometrial samples of FSH+HCG-treated women compared with FSH+LH-treated women (p<0.0001; Student T test). Endometrial samples presented a low percentage of CD14+ and CD3+ cells, with no significant differences between the three cohorts of women (p=0.74; p=0.123; Student T test). When we looked at CD3+ cell subsets, we observed a decrease in $CD4^{+}CD25^{+}CD127^{dim/-}$ regulatory T cells in FSH+HCG-treated women in comparison with FSH+LH-treated women.

Limitations, reasons for caution: The main limits of this project are two: i) the small number of enrolled subjects and ii) monocentric recruitment. Validation of these data will require a larger multicentric study.

Wider implications of the findings: The present data suggest that, by influencing the expression of biomarkers of endometrial receptivity, alternative gonadotrophin regimens may differently impact on implantation. This leads to the hypothesis of the development of personalized gonadotrophins IVF treatments aimed at improving endometrial receptivity.

Trial registration number: NA

P-680 Comparable outcome using Oral dydrogesterone versus micronized vaginal progesterone (MVP) in aFET (artificial frozen embryo transfer)

Y. Atzmon[1], N. Aslih[1], M. Michaeli[1], A. Bilgory[1], E. Shalom-paz[1]

[1]Hillel - Yaffe medical center, ivf, Hadera, Israel

Study question: Does using oral dydrogesterone instead of micronized vaginal progesterone (MVP) in aFET impact pregnancy outcome
Summary answer: Oral dydrogesterone showed non-inferiority to MVP in aFET in terms of chemical and clinical pregnancy outcome
What is known already: During endometrial preparation for FET in artificial cycle the most common progesterone used is MVP in forms of vaginal suppository or as vaginal gel. Both are known for their inconvenient use, vaginal discharge and irritation. During 2017 two RCT's were conducted comparing dydrogesterone and MVP in fresh IVF cycles and oral dydrogesterone was found to have non-inferiority results in pregnancy outcome. Since then, we faced a growing demanded raised from the patients to use oral progesterone in aFET.
Study design, size, duration: Retrospective cohort study between 2018 to 2019. All patients used oestradiol 6-10 mg a day for at list 8 days as priming endometrial growth. For progesterone exposure, one of the following medications was supplemented-1. MVP 300 mg daily, 2. 8% MVP gel 90 mg daily. 3. Oral dydrogesterone (10 mg X3 TID).

Two groups were compared- **group A**- oral dydrogesterone support vs. **group B**-MVP supplementation.
Participants/materials, setting, methods: In total 238 cycles included in **group A** and 381 cycles in **group B** were analyzed. Cleavage stage embryos and blastocyst embryos were transferred after exposing to progesterone according to the established protocols. Clinical pregnancy was the primary endpoint.
Main results and the role of chance: Both groups were comparable in terms of age, BMI, and cause of infertility, gravidity and parity, maximal endometrial thickness. Although Embryo's quality scoring was significantly better in group B 2.16±0.79 vs. 2.45±0.78; P=0.02, the chemical pregnancy, and clinical pregnancy rates were similar in both groups 42.6% vs. 43.1(P=1) and 35.1% vs. 37.4% (P=0.85) respectively.
Limitations, reasons for caution: The limitation of the study are based in the retrospective nature of it and the heterogeneity of the cohort
Wider implications of the findings: Safety was shown in the use of dydrogesterone in RPL and fresh IVF cycles. Prospective studies reported non-inferiority in fresh IVF cycles (Lotus 1,2) for luteal support. This study reports, for the first time, the effectiveness of dydrogesterone in aFET cycles. More RCT's are needed to establish our results.
Trial registration number: not applicable

P-681 The relationship between the number of supplementary blastocysts available and live birth rate following single embryo transfer : analysis of 10 015 fresh IVF cycles

Y. Beebeejaun[1], T. Copeland[2], L. Polanski[3], T. El Toukhy[3]

[1]King's College London, Department of Women's Health, London, United Kingdom ;
[2]University of California Los Angeles, Department of Health Policy & Management, Los Angeles, U.S.A. ;
[3]Guy's and St Thomas' Hospital, Assisted Conception Unit, London, United Kingdom

Study question: Does the number of supplementary blastocysts available for cryo-storage affect the live birth rate in a fresh IVF cycle?
Summary answer: The present study demonstrated a non linear association between the number of supplementary blastocysts and live birth rate in different age groups.
What is known already: The ability to predict the likelihood of a live birth after single fresh embryo transfer is important for treatment planning and managing patient expectation, particularly in their first in vitro fertilisation (IVF) cycle. Cryopreservation of supernumerary embryos is often regarded as an important prognostic variable and a surrogate marker of success. Previous large studies have examined the association between the number of oocytes retrieved and cleavage-stage embryos available, and the odds of a live birth following a fresh embryo transfer, whereas the relationship between the number of supernumerary blastocysts cryopreserved following a fresh embryo transfer has not been rigorously studied.
Study design, size, duration: Anonymised data on first IVF/ICSI cycles performed at Guy's and St. Thomas' Hospital in London, were prospectively collected between July 2006 and June 2018. Cycles involving pre-implantation genetic testing (PGT), oocyte donation, transfer of cleavage-stage or more than one fresh embryo or total embryo freezing were excluded. Since age is known to be associated with lower live birth rates, sub-analysis was performed for women aged <35 years, 35-39 years and > 40 years old.
Participants/materials, setting, methods: Cycle data on women undergoing IVF/ICSI treatment between 2006-2018 were analysed to compare clinical outcomes of live birth rate based on the number of supplementary embryos obtained and stratified by the pre-defined age groups.
A multivariate logistic regression analysis was used to examine the association between livebirth ans supernumerary blastocysts after controlling for confounders. Fractional logistic regressions were modelled using a cubic polynomial transformation of the number of supernumerary blastocysts and stratified by age
Main results and the role of chance: Cycles with ≥1 supernumerary blastocysts cryopreserved had a significantly higher live birth rate compared to those without blastocyst cryopreservation (38.5%, 95% CI 36.9-40.0% vs. 24.3%, 95% CI 23.3-25.5; P<0.0001) and adjusted odds ratio (OR) 1.76, 95% confidence interval (CI) 1.61-1.92, P<0.0001).

The likelihood of having a live birth, **when < 35 years old**, increased linearly from 0.33(95% CI 0.31–0.34; P<.0001 to 0.80 (95% CI 0.74–0.86; P<.0001) between 1-6 blastocysts cryopreserved. A non-linear association was then seen up to 0.95(95% CI 0.92–0.97; P<.0001) if ≥10 blastocysts were cryopreserved.
In the 35-39 age group, the likelihood of having a live birth increased linearly from 0.30 (95% CI 0.28–0.32; P<.0001) to 0.82 (95% CI 0.73–0.91; P<.0001) for up to 6 blastocysts and non-linearly to 0.96 (95% CI 0.93–0.99; P<.0001) if ≥10 blastocyst were cryopreserved.
When aged 40 years and above, the likelihood of having a live birth increased linearly from 0.26 (95% CI 0.19–0.32; P<.0001) to 0.83 (95% CI 0.68–0.97; P<.0001) if 4 blastocysts were cryopreserved and non-linearly to 0.99 (95% CI 0.98–0.99; P<.0001) if >10 blastocysts were cryopreserved.
Limitations, reasons for caution: Limitations with observational data would apply to this study including residual confounding.
Wider implications of the findings: The predictability of a live birth with regards to the number of blastocysts available following IVF is of clinical relevance for patients and clinicians.
Trial registration number: Not applicable

P-682 Whole-genome microRNA expression profiles from single pre-ovulatory follicles of oocyte donors and polycystic ovarian syndrome (PCOS) patients

I. Rooda[1,2], M.M. Hasan[3], K. Roos[1,4], J. Viil[3], O.P. Smolander[1], A. Salumets[2,3,5,6], A. Fazeli[3,7], A. Velthut-Meikas[1]

[1]Tallinn University of Technology, Department of Chemistry and Biotechnology, Tallinn, Estonia ;
[2]Competence Centre on Health Technologies, Competence Centre on Health Technologies, Tartu, Estonia ;
[3]University of Tartu, Department of Pathophysiology, Tartu, Estonia ;
[4]Nova Vita Clinic, Nova Vita Clinic, Tallinn, Estonia ;
[5]University of Tartu, Department of Obstetrics and Gynecology, Tartu, Estonia ;
[6]University of Helsinki and Helsinki University Hospital, Department of Obstetrics and Gynaecology, Helsinki, Finland ;
[7]University of Sheffield, Department of Oncology and Metabolism, Sheffield, United Kingdom

Study question: How do miRNA expression profiles of follicular fluid (FF), extracellular vesicles (EVs) and granulosa cells from single follicles differ between polycystic and healthy ovaries?
Summary answer: It is possible to successfully sequence small RNA from low input material like FF, EVs and cells of individual follicles.
What is known already: Several miRNAs have been shown to differ between women with PCOS and control group. Moreover, miRNAs are considered as potential diagnostic markers and therefore may reveal important differences in cell-to-cell signaling in PCOS women compared to healthy individuals. miRNAs regulate gene expression in the cells where they are transcribed, but they can also be secreted into extracellular space. One mechanism to secrete miRNAs is to pack them into EVs. Analyzing cellular and extracellular miRNAs of follicles can provide information about the origin of the miRNAs and cell-to-cell signaling differences between PCOS women and oocyte donors.
Study design, size, duration: Genome-wide miRNA expression profiles were obtained by small RNA sequencing technique. miRNAs were sequenced from FFs, EVs and cells collected from the FF of individual follicles. Material for sequencing

were obtained from patients undergoing ovarian stimulation and ovarian puncture. Two patient groups were formed: women with diagnosed PCOS and oocyte donors as a control group. Eight women were recruited per group.
Participants/materials, setting, methods: FF and cellular material were collected from the first aspirated follicle visibly clear of blood contamination. Starting amount for miRNA library preparation was 500µl of FF, EVs extracted from 500µl of FF, and 10 ng of small RNA fraction from cells. RNA was extracted with miRNEASY micro kit (QIAGEN) and miRNAs libraries prepared with QIAseq miRNA Library Kit (QIAGEN). Sequencing was performed on NextSeq 500 platform (Illumina) with single-end reads of 75bp length.
Main results and the role of chance: In previous studies miRNA sequencing technique has been used for pooled FF samples. Development in the area of miRNA library preparation methods has made possible to sequence low input material like small volumes of FF and miRNAs extracted from EVs. Our preliminary sequencing results showed that five hundred µl of FF collected from a single follicle was enough to obtain sequences of over 300 different miRNAs. Principal component analysis showed that EV and FF samples from the same individual follicle clustered separately. Therefore, not all miRNAs present in whole FF are packed into EVs. To obtain more information about miRNA signalization of individual follicles we added miRNAs from granulosa cells into data analysis. Comparing miRNA profiles from follicular cells, FF and EVs provides information about miRNAs that are secreted into follicular environment by follicular cells and used for cell-to-cell signalization.
Limitations, reasons for caution: Sequencing low input material have its limitations, miRNAs with very low expression levels probably do not reach the detection levels. Only one follicle per woman was analyzed and the group size is relatively small.
Wider implications of the findings: RNA sequencing from single follicles will give an opportunity to evaluate individual follicle environment and enable to reveal differences between follicles developed in polycystic ovaries compared to healthy follicles. Moreover, this approach may provide new information about the developmental potential of oocytes from individual follicles.
Trial registration number: not applicable

P-683 Clinical outcomes after in vitro maturation (IVM) cycles in patients with polycystic ovaries before and after government legislation.

"Abstract withdrawn by the authors"

P-684 Advanced oxidation protein products induce G1/G0 phase arrest in ovarian granulosa cells via a ROS-JNK/p38 MAPK-p21 mediated pathway

Z. Xingyu[1], C. Shi-Ling[1], Z. Jun[1], L. Ying[1], C. Ying-Xue[1], Z. Jing[1], Z. Qing-yan[1]

[1]Nanfang Hospital- Southern Medical University, the Center for Reproductive Medicine- Department of Gynecology and Obstetrics, Guangzhou, China

Study question: What are the effect and mechanism of advanced oxidation protein products (AOPPs) on the cell cycle of ovarian granulosa cells.
Summary answer: AOPPs arrest ovarian granulosa cell cycle progression in G1/G0 phase by reducing cyclin E and CDK2 expression through ROS-JNK/p38 MAPK-p21 signaling.
What is known already: AOPPs, novel protein markers of oxidant-mediated protein damage, have been found to be implicated in polycystic ovarian syndrome (PCOS) and endometriosis (EMT). However, what is the role of AOPPs played on the ovarian granulosa cells is unclear. Recently, the accumulation of AOPPs has been reported to contribute to cell cycle arrest in intestinal epithelial cells and hepatocytes.
Study design, size, duration: KGN cell line was tearted with different doses of AOPPs for 24h to measure the cell cycle distribution and the protein levels of cell cycle relative proteins, and for 2h to measure the levels of ROS. KGN were also treated with 200µg/mL AOPPs for 3h to measure total and phosphorylated protein levels of JNK and p38 MAPK. Thirty-six female SD rats were randomly divided into four groups: PBS, bovine serum albumin (BSA), AOPPs, and AOPPs+NAC.
Participants/materials, setting, methods: The cell cycle distribution were measured by flow cytometry. Western blotting analysis was used to measuring the protein levels of cycle E, CDK2, p21, total and phosphorylated JNK/p38 MAPK. Intracellular ROS was detected by the probe DCFH-DA. Fluorescence intensity was measured on a SpectraMax M5 system. All experiments were repeated at least 3 times. Differences in variables between groups were determined via independent sample t-tests.
Main results and the role of chance: KGN treatment with AOPPs were arrested in G1/G0 phase, and markedly reduced the expression of cyclin E and CDK2 and increased the expression of p21, whereas native BSA had no effect. AOPPs administration significantly increased intracellular ROS generation. We also found that AOPPs induced KGN G1/G0 phase arrest by phosphorylation of JNK and p38 MAPK. Furthermore, the pretreatment of NAC (ROS scavenger), si-P21, si-JNK and si-p38 MAPK resulted in a decreased expression of p21 and increased expression of cyclin E and CDK2. AOPPs-induced G1/G0 phase arrested was also significantly blocked by the pretreatment of NAC (ROS scavenger), si-P21, si-JNK and si-p38 MAPK. In SD rats, AOPPs treatment reduced the protein expression of cyclin E and CDK2 in ovary, and increased the expression of p21, phosphorylated JNK and p38 MAPK.
Limitations, reasons for caution: This study was conducted using a cell model and rat model. Thus, this finding may not directly represent human.
Wider implications of the findings: Our findings reveal that AOPPs influence ovarian granulosa cell cycle progression by reducing cyclin E and CDK2 expression through ROS-JNK/p38 MAPK-p21 signaling. Consequently, AOPPs may represent a potential therapeutic molecule of many ovarian disease, included PCOS and EMT.
Trial registration number: not applicable

P-685 Serum progesterone level on frozen embryo transfer day is lower with hormonal therapy than with a natural cycle, a retrospective single university centre study

O. Pouget[1], M. Alsawaf[1], I. Zuna[1], M. Bonneau[1], M.L. Tailland[1], S. Ripart[1], S. Huberlant[1]

[1]CHU Nimes, Gynecology Obstetric, Nimes, France

Study question: Determine whether a difference in serum progesterone level on the transfer day exists according to the type of endometrial preparation for patients benefiting from a frozen embryo transfer
Summary answer: Serum progesterone level on frozen embryo transfer day is lower with endometrial preparation with hormonal therapy than with a natural cycle
What is known already: Live birth rate after embryo transfer with natural cycle or hormonal therapy seems to be comparable, but the pregnancy loss rate is higher with hormonal therapy. This high pregnancy loss rate may be due to luteal insufficiency with endometrial preparation with hormonal therapy, characterized by a low level of serum progesterone in the luteal phase
Study design, size, duration: We conducted a retrospective study from may to december 2019 including 115 embryo transfers (47 with a natural cycle and 68 with hormonal therapy).
All couples undergoing a frozen embryo transfer in the university centre of Nimes (France) were included
Participants/materials, setting, methods: We compared 2 endometrial preparations : natural cycle (with or without stimulation by FSH or hMG) and hormonal therapy.
The main outcome data was the level of serum progesterone (ng/mL) on the day of frozen embryo transfer, endometrial thickness, pregnancy rate, pregnancy loss rate, maternal characteristics, and embryo characteristics.
We compared quantitative measures using the Mann-Whitney-Wilcoxon test and qualitative variables using the Fisher test with the help of the Statistic software R
Main results and the role of chance: Mean serum progesterone level on embryo transfer day was 25.47 ng/mL after natural cycle versus 14.32 ng/mL after hormonal therapy (p <10-8).
There was no significant difference for demographic and fertility data (age, type of embryo, type of infertility, basal FSH, LH, Estradiol and AMH levels), endometrial thickness, number and type of embryo transferred, age of infertility, pregnancy rate and pregnancy loss rate. Body mass index was 22.9 kg/m2 in natural cycle group vs 24.8 kg/m2 in hormonal therapy group (p=0.03).
No difference was found in serum progesterone level between the progressive pregnancy and pregnancy loss (respectively 17.48 ng/mL vs 20.82 ng/mL, p=0.7)
Limitations, reasons for caution: To analyze the fetal loss rate, our workforce is small (22 progressive progressive pregnancy and 12 pregnancy loss)

Wider implications of the findings: Further research is necessary to determine if this difference on serum progesterone level has any relation with higher pregnancy loss rate with hormonal therapy
Trial registration number: not applicable

P-686 Differential gene expression in mouse oocytes from primordial and primary follicles from cultured and non-cultured ovaries

M. Amoushahi[1], K. Lykke-Hartmann[1,2,3]

[1]Aarhus University, Department of Biomedicine, Aarhus, Denmark ;
[2]Aarhus University, Department of Clinical Medicine, Aarhus C, Denmark ;
[3]Aarhus University Hospital, Department of Clinical Genetics, Aarhus, Denmark

Study question: Does comparative transcriptomic analysis on isolated oocytes from mouse primordial and primary follicles of cultured and non-cultured ovaries, prepare a better insight into molecular mechanisms involved in ovarian follicular activation?
Summary answer: Comparative transcriptomic analysis on oocytes from primordial and primary follicles from cultured and non-cultured ovaries, advanced our findings of molecular mechanisms involved in follicular activation.
What is known already: Different signaling pathways such as, PI3K/AKT are identified during ovarian primordial follicle activation.
Study design, size, duration: We compared oocytes from primordial (n= 277) and primary (n= 170) follicles in removed ovaries from 14 days old mice (*in-vivo* group) and oocytes from primordial (n= 299) and primary (n=250) follicles in cultured ovaries from 7 days old mice after 7 days (*in-vitro* group).
Participants/materials, setting, methods: ovaries from 7-days old female mice were cultured for 7 days. Non-cultured and cultured ovaries fixed, dehydrated, embedded and sectioned. Oocytes of primordial and primary follicles in both groups were isolated by Laser Capture Microdissection, and subjected to RNA extraction. Extracted RNA were sequenced using Illumina HiSeq platform. Quality filtering, trimming and removing adaptor sequences of raw data were performed. Expression value normalization and comparisons were performed using DESeq2 in software R.
Main results and the role of chance: Differential expressed gene analysis in this study, showed 1258 significant genes with 976 up-regulated genes and 282 down-regulated genes in oocytes of primordial follicles from in vitro group. Moreover, 962 and 1309 Genes were up-regulated and down-regulated respectively, in oocytes of primary follicles from in-vitro group compared to in-vivo group. *PIK3* was significantly up-regulated in oocytes of primary follicles from in-vitro group compare to in-vivo group. However, most of identified genes in primordial follicle activation were not significantly up or down-regulated in comparison between groups. Moreover, there were not significant difference between expression of oocyte specific genes such as, *NOBOX*, *SIX6*, *BSX* and *DMRTC2* in oocytes of primordial and primary follicles derived from non-cultured and cultured ovaries. Inhibitory genes in primordial follicles activation, such as *PTEN*, *P27*, *FOXO3* and *GSK* were upregulated in oocytes of primordial follicles and down-regulated in oocytes of primary follicles. These results confirmed that there is a movement in gene expression during primordial follicle activation.
Limitations, reasons for caution: This is a descriptive analysis and functional studies were not performed.
Wider implications of the findings: For the first time, we performed comparative transcriptomic analysis in mouse oocytes derived non-cultured and cultured ovaries. These descriptive results can prepare a better understanding of molecular mechanism during ovarian follicular development.
Trial registration number: not applicable

P-687 Serum luteinizing hormone level on hCG trigger day does not associate with cumulative live birth after IVF/ICSI with GnRH-agonist long protocol

R.H. Wu[1], Y.X. Wen[1], L. Luo[1], Y.B. Li[1], M.H. Chen[1]

[1]The First Affiliated Hospital of Sun Yat-sen University, Reproductive medicine center, Guangzhou-Guangdong, China

Study question: Does the serum luteinizing hormone(LH) level on hCG trigger day affect the cumulative live birth after IVF/ICSI with GnRH-agonist long protocol?
Summary answer: Serum LH level on hCG trigger day does not associate with cumulative live birth or embryo quality after IVF/ICSI with GnRH-agonist long protocol.
What is known already: Whether supplement of exogenous LH is beneficial to IVF outcome is still controversial.Serum LH level reflect the result of addition of exogenous LH on the basis of endogeneous LH level.It is unclear whether serum LH level is associated with cumulative live birth(CLBR) after IVF/ICSI.
Study design, size, duration: This is a retrospective cohort study.A total of 5734 women(6036 ovarian stimulation cycles) who underwent IVF/ICSI with GnRH-agonist long protocol at our hospital from January 2013 to December 2017 were identified and reviewed.
Participants/materials, setting, methods: The patients were assigned to three groups according to the serum LH levels on hCG trigger day: >90th, 10th-90th and <10th.CLBR was defined as the first live birth per ovarian stimulation cycle including fresh and frozen cycles and evaluated by group.Multivariable linear regression and binary logistic regression was used to assess the association between LH levels on hCG trigger day and embryo quality,CLBR after adjusting confounding factors.
Main results and the role of chance: The cumulative live birth rate was 67.6%, 63% and 58.6% with the serum LH levels on trigger day<0.39,0.39-1.54 and >1.54 mIU/ml during ovarian stimulation with GnRH-agonist long protocol respectively(P=0.043).However,the serum LH level on trigger day was not associated with cumulative live birth(OR=1.598,95%CI 0.870-2.936,P=0.13) after adjusting female age,duration of infertility,body mass index,the number of gravidity,antral follicle count,basal FSH level,total dosage of gonadotropin,addition of HMG,the thickness of endometrium and FSH,estrogen,progesterone level on the hCG trigger day,retrieved oocytes,mature oocytes,the number of viable embryo and high-quality embryo.The number of viable embryo and high-quality embryo decreased with the increase of serum LH levels on trigger day,but the serum LH level on trigger day was not associated with the number of viable embryo(B=0.032,P=0.474) and high-quality embryo(B=-0.011,P=0.802) after adjusting female age,duration of infertility,FSH and progesterone Level on day of hCG administration,total dosage of gonadotropin and the number of mature oocyte.
Limitations, reasons for caution: As a retrospective study,our analysis depended on previously recorded data.Therefore,certain variables such as complications could not be collected and conclusion is limited to achieve one live birth in each stimulation cycle.
Wider implications of the findings: The serum luteinizing hormone level on hCG trigger day does not affect the possibility to achieve one live birth per ovarian stimulation cycle,and supplement of exogenous LH may not benefit IVF/ICSI outcome.
Trial registration number: 81601239

P-688 The ratio AMH/antral follicle count an innovative tool for assessing the follicular health in young women with diminished ovarian reserve

C. Lethielleux[1], C. Sonigo[2], C. Vinolas[1], I. Cedrin-Durnerin[1], M. Grynberg[2]

[1]Jean Verdier Hospital, Reproductive Medicine and Fertility preservation, Bondy, France ;
[2]Hopital Antoine Béclère, Reproductive Medicine and Fertility preservation, Clamart, France

Study question: Does diminished ovarian reserve (DOR) and its etiology impact the AMH/AFC ratio?
Summary answer: AMH/AFC ratio varies according to the etiology of DOR in young women, suggesting different impact on the follicular health, and further oocyte quality
What is known already: Anti-Müllerian hormone and antral follicle count currently represent the two most accurate markers of the follicular ovarian status. Even though they may diagnose a reduction in the follicular stockpile, low values remain inefficient for predicting poor oocyte quality, in particular in young women. Since AMH is produced by the granulosa cells of follicles ranging from primary to small antral follicles, we hypothesized that the etiology of diminished ovarian reserve might differently impact the follicular health and their capacity of producing this peptide.

Study design, size, duration: From November 2018 to December 2019, we conducted a monocentric, retrospective study including a total of 195 infertile patients <37 years with DOR.
Participants/materials, setting, methods: All patients underwent measurement of AMH levels and AFC. DOR was diagnosed according to the Bologna criteria (AMH<1.1 ng/mL and AFC<7). AMH/AFC ratio was compared to values obtained in 53 tubal or male infertility patients matched for age and BMI, with AMH and AFC in the normal ranges. This ratio was studied according to the etiology of DOR: genetic (n=9), post-chemotherapy (n=41), idiopathic (n=87) or ovarian diseases (ovarian cyst or history of ovarian surgery, n=58).
Main results and the role of chance: Overall, median age of women with DOR was 31 (18-37.5) years. As expected, age and BMI were comparable in women with DOR and those having normal ovarian reserve tests. In addition, the AMH/AFC ratio failed to show any difference between these 2 groups (0.136 ± 0.26 vs. 0.168 ± 0.08, NS, respectively). Among women with DOR, the etiology was significantly associated with different AMH/AFC ratio. Indeed, patient with DOR of "mechanic" origin (ovarian diseases group) displayed higher mean values (0.235 ± 0.41 ng/mL/ Foll) when compared with those included in genetic (0.082 ± 0.12 ng/mL/ Foll), idiopathic (0.101 ± 0.11 ng/mL/ Foll) or post-chemotherapy (0.085 ± 0.20 ng/mL/ Foll) groups. Moreover, genetic and post-chemotherapy DOR was also associated with lower AMH/AFC ratio in comparison with idiopathic DOR.
Limitations, reasons for caution: Despite interesting results, the presents study remains retrospective on a limited number of patients. In addition, AMH/AFC ratio constitute an indirect method for assessing the follicular health.
Wider implications of the findings: AMH/AFC ratio is an innovative tool aiming to indirectly assess follicular health and possibly oocyte quality in young women with DOR. The etiology of DOR differently impacts the follicular function as reflected by AMH/AFC ratio. Therefore, data on live birth rates following natural or medically assisted pregnancies is needed.
Trial registration number: not applicable

P-689 Effect of two different doses of Vitamin D supplementation on clinical, metabolic and hormonal profiles of Insulin-resistant PCOS patients: A Randomized Controlled Trial

A. Bahadur[1], A. Yadav[1], J. Chaturvedi[1], R. Mundhra[1], R. Rajput[1], M. Naithani[2], N. Bhattacharya[1], J. Prerna[1], S. Kumari[1], N. Verma[1], M. Kumawat[1], P. Kumari[1]

[1]All India Institute of Medical Sciences, Obstetrics & Gynaecology, Rishikesh, India ; [2]All India Institute of Medical Sciences, Biochemistry, Rishikesh, India

Study question: To compare the effect of two different doses of Vitamin D Supplementation on clinical, metabolic and hormonal profiles of insulin resistant PCOS cases
Summary answer: This study supports beneficial effects of high dose vitamin D supplementation along with metformin on clinical, metabolic and hormonal status in insulin sensitive PCOS women.
What is known already: Insulin resistance plays a key role in reproductive and metabolic dysfunctions in PCOS. The rationale for vitamin D supplementation in PCOS women is based on the fact that vitamin D supplementation decreases Insulin Resistance by its role on glucose metabolism. Data on the effects of vitamin D supplementation along with metformin usage on metabolic status of patients with polycystic ovary syndrome (PCOS) are scarce.
Study design, size, duration: Prospective, open label, non-blinded randomized controlled trial conducted in department of Obstetrics and Gynecology, All India Institute of Medical Sciences, Rishikesh, India

110 women with PCOS were screened. 24 patients with BMI > 30 kg/m^2 and 14 were not willing to participate in study were excluded.

72 women with PCOS and Insulin Resistance in age group 20-35 years were included for a period of 20 months, underwent biochemical and hormonal assessment on day 2 of cycle
Participants/materials, setting, methods: Patients in Group I (n=36) received Tablet Metformin 500 mg twice a day orally along with Tablet vitamin D3 1000 IU orally for 3 months. Patients in Group II (n=36) received Metformin 500 mg twice a day orally along with Tablet vitamin D3 4000 IU orally for 3 months
Primary outcome: Change in HOMA-IR after treatment in both groups
Main results and the role of chance: Vitamin D supplementation (4000 IU) resulted in beneficial effects on HOMA-IR, mFG score, global acne score, menstrual cycle regularity, BMI. LH levels, triglyceride levels, DHEAS values, fasting and postprandial blood sugars, fasting and postprandial insulin levels as compared to 1000 IU vitamin D supplementation. ($p < 0.05$). The change in mFG score over 3 months in Group I was -1.19 ± 2.67 and in Group II was - 2.81 ± 4.15. There was statistically significant difference between the change in mFG score in the two groups (p=0.027) with the decrease in Group II being more than Group I.

Menstrual cycle irregularity in Group II was 31(86.11%) at baseline and decreased to 21(58.3%) at 3months.There was statistically significant decrease in women having menstrual irregularity between baseline and at 3 months in both the Groups with p value in Group I of 0.012 and in Group II of 0.018. The change in Vitamin D levels (ng/ml) at 3 months in Group I was 5.36 ± 2.79 and in Group II was 13.19 ± 3.26. There was statistically significant difference between rise in Vitamin D levels (ng/ml) in two groups ($p < 0.0001$) with the rise in group II being more than Group I.
Limitations, reasons for caution: Further studies with larger sample size are required before conclusion can be derived that combination of vitamin D3 4000 IU with metformin is better than vitamin D3 1000 IU with metformin in improving the clinical, hormonal and metabolic parameters in PCOS patients.
Wider implications of the findings: Hypovitaminosis D is very common in PCOS patients and exacerbates the metabolic abnormalities. It is essential to screen all the PCOS patients for
vitamin D (25OHD) deficiency and institute appropriate replacement therapy to decrease insulin resistance and thus the metabolic, hormonal and clinical profile of PCOS patients.
Trial registration number: This trial was Registered with the Clinical Trial Registry of India (CTRI) CTRI/2019/11/021926

P-690 The role of follicular Anti-Mullerian hormone in woman undergoing IVF/ICSI with regard to size, oocyte, sociodemographic parameters and other hormones and vitamins

V. Ziller[1], L. Witzke[1], M. Nesbigall[1], P. Pfefferle[2], B. Greene[3], U. Wagner[1]

[1]University Medical Center Giessen and Marburg, Department of Gyn. Endocrinology and Reproductive Medicine, Marburg, Germany ; [2]University Medical Center Giessen and Marburg, Comprehensive Biomaterial Bank Marburg CBBMR, Marburg, Germany ; [3]University Medical Center Giessen and Marburg, Biometrics, Marburg, Germany

Study question: Do levels of AMH in separately analysed follicles differ depending on size of the follicle and is it associated with steroid production and ART outcome?
Summary answer: Larger follicles do not produce more fAMH than smaller ones. The level of fAMH is correlated to steroid levels and the presence of an oocyte.
What is known already: The value of serum Anti-Mullerian hormone (AMH) as a marker for the ovarian reserve has been well established. However, the presence and actions of AMH on the follicular level has not yet been sufficiently clarified. Therefor the aim of this study was to investigate how AMH concentrations present in terms of follicle size, presence of an oocyte, demographic data and ART outcome. We also explored the association of fAMH with other hormones and vitamins such as Vitamin D, Luteinizing hormone (LH) and steroids in follicular fluid on the basis of separated follicles.
Study design, size, duration: In this prospective exploratory study, infertile women undergoing IVF between January 2018 until December 2019 were included. Written permission was required. Known illnesses of the reproductive system and infectious diseases were defined as exclusion criteria. A total of 61 female fertility patients undergoing IVF or ICSI therapy and 376 separated follicles were included.
Participants/materials, setting, methods: On the day of oocyte retrieval we collected the FF of each follicle separately and also a serum sample. The samples were individually analyzed regarding their AMH level and other hormone concentrations. The follicles were divided into a large and a small group according to their size (large (≥16mm), small<16mm)). It was also documented whether

the follicle contained an oocyte or not. Demographic data and ART outcome was added from patients records.

Main results and the role of chance: We investigated the follicular fluid AMH (fAMH) and serum AMH (sAMH) of 61 female fertility patients undergoing IVF or ICSI therapy (fAMH n= 376, sAMH n=44).

Follicles with a large diameter contained a significantly lower concentration of fAMH (mean difference around 1 ng/ml les, p=0.001). In addition, follicles that contain an oocyte showed a significantly higher fAMH level than those without one (p<0.001). There was a significant but weak correlation between fAMH and sociodemographic parameters such as patients age (r=-0.11, p<0.001) and BMI (r=0.03, p<0.006). Follicular AMH was strongly correlated to diverse other parameters such as Vitamin D (r=-0.13, p<0.001), LH (r=-0.35, p<0.001) and progesterone (r=-0.21, p<0.001) in the corresponding follicle. As expected, there is a significant positive correlation between fAMH and sAMH (r=-0.42, p<0.001)

Limitations, reasons for caution: The process of follicle separation is not 100% exact, however an in-house pre-study using predefined test solutions underlined reliability (data on file). IVF stimulated cycles may not represent the situation in natural cycles and further studies are needed.

Wider implications of the findings: As AMH and reproduction is an important part of current medical and scientific discussions correlation actual findings with clinical outcome parameters, such as fertilization rate, pregnancy rate etc. are planned to gain further insights in the mechanisms of follicular AMH and its role in assisted reproduction.

Trial registration number: n/a

P-691 The use of follitropin delta for ovarian stimulation for in vitro fertilization in patients with a low ovarian reserve: results from clinical practice.

E.C. Creutzberg[1], P. Wijngaard-Boom[2], T. Stijnen[3], J. Laven[2]

[1]Ferring BV, Gynaecology, Hoofddorp, The Netherlands ;
[2]Erasmus Medical Centre, Reproductive Medicine, Rotterdam, The Netherlands ;
[3]Leiden University Medical Centre, Biostatistics, Leiden, The Netherlands

Study question: This study describes in a real life setting the influence of a higher dose of follitropin delta in patients with a low ovarian reserve.

Summary answer: Increasing the dose of follitropin delta in patients with low ovarian reserve did not lower cancellation rates and did not result in improved clinical outcome.

What is known already: In pivotal trials performed with follitropin delta, median Anti-Mullerian Hormone (AMH) was 16.3 pmol/l. The general IVF population in the Erasmus University Medical Centre (EUMC) consists of patients with a wide range of AMH-values, including patients with low levels who are at risk for under response leading to cycle cancellation. Current evidence on ovarian response and treatment outcome in patients with low ovarian reserve shows no improvement of ongoing pregnancy rates with increased dose of FSH in women with low ovarian reserve, despite a lower cancellation rate and a higher number of oocytes retrieved.

Study design, size, duration: Since its introduction in EUMC in 2017, 148 patients with AMH lower than 7.14 pmol/L were treated with follitropin delta. Due to a relatively high percentage of cancellations, after the first half year and up to and including September 2019, patients with low to very low AMH levels were treated with a starting dose higher than calculated by the approved dosing algorithm. The outcomes of the first IVF cycle including frozen embryo transfers are described.

Participants/materials, setting, methods: AMH was measured using the automated Elecsys® AMH plus immunoassay (Roche Diagnostics International). Among the 148 consecutive patients with low ovarian reserve (AMH<7.14pmol/l), the first 47 patients were treated with 12 µg/day (as per dosing algorithm), and the next 101 patients were treated with a higher starting dose of 18 to 24 µg/day. All treatment results were collected from the clinical records. Data were described as mean (SD) and statistically analyzed using t-test.

Main results and the role of chance: The 47 patients that received a follitropin delta dose of 12 µg compared to the 101 that received a dose of 18 or 24 µg had mean (SD) age of 36.2 (4.0) vs 37.1 (4.1) years, an AMH of 4.5 (1.6) versus 3.2 (2.1) pmol/l and a body weight of 69.3 (13.7) vs 68.8 (13.3) kg. The percentage of canceled cycles due to insufficient number of follicles ≥11mm at day of last ultrasound dropped non-significantly from 34.0% in the standard-dosed group to 22.8% in the higher-dosed group (p=0.15). The number of oocytes (5.1 (3.5) vs 4.8 (2.8)) and cleavage stage embryos (2.8 (2.2) vs 2.6 (2.3)) did not change significantly. Ongoing pregnancy rate after 12 weeks including frozen embryo transfers was 17.4% in the standard-dosed group and 13.3% in the higher-dosed group (p=0.50).

Limitations, reasons for caution: This is an analysis of the use of follitropin delta in patients with low ovarian reserve on the base of AMH level in clinical practice. A heterogeneous population with different phenotypes was treated, in which the patients were not randomized and no control group was applied.

Wider implications of the findings: These preliminary results indicate that applying a dose of follitropin delta higher than calculated did not result in a lowering of cancellation rates or improvement in clinical outcome. Therefore it seems important not to deviate from the dosing algorithm and manage expectations in these patients with poor ovarian response.

Trial registration number: not applicable

P-692 The association of FSH and AMH basal levels with live birth rates after ICSI/IVF is age dependent

J. Buratini[1,2], M. Dal Canto[1], F. Brambillasca[1], C. Brigante[1], S. Gippone[1], E. De Ponti[3], M.M. Renzini[1]

[1]Biogenesi - Reproductive Medicine Centre, Istituti Clinici Zucchi, Monza, Italy ;
[2]Sao Paulo State University, Institute of Biosciences, Department of Physiology ;
[3]ASST Monza, Medical Physics, Monza, Italy

Study question: Does the association of AMH and FSH basal levels with live birth rates following ICSI/IVF change with maternal age?

Summary answer: AMH is more strongly associated with live birth than FSH in patients > 35 years, whereas the opposite is observed in patients ≤ 35 years.

What is known already: FSH and AMH have been investigated as predictors of IVF/ICSI outcomes. The literature suggests that AMH has a higher predictive value, although each alone can only weakly predict live birth. The inter-cycle variability of FSH basal levels increase with age, compromising the predictive performance of FSH in advanced maternal ages. In contrast, AMH levels are more strongly correlated with live birth in patients >35 years. Although FSH and AMH levels are often inversely correlated, 25-40% of the patients present discordant values. No study so far has assessed the impact of maternal age on AMH and FSH predictive values.

Study design, size, duration: Retrospective consecutive cohort of 2830 first ICSI/IVF cycles from 2016 to 2019. Patients were stratified according to AMH and FSH cut-offs (1 ng/mL for AMH, 10 IU/L for FSH) into four groups: A (AMH>1/FSH≤10); B (AMH>1/FSH>10); C (AMH≤1/FSH≤10) and D (AMH≤1/FSH>10). Live birth rates after the first ET were compared among groups and the correlation of AMH and FSH values with live birth rate was assessed in three populations: total, ≤35 and >35 years old.

Participants/materials, setting, methods: Women aged 20 to 45, with FSH levels measured on menstrual cycle day 2 and AMH on any day underwent COS, OPU and ICSI or IVF. One to three embryos were transferred fresh on D+3. Live birth rates (LBR) were compared among AMH/FSH groups with the Chi-square test. The correlation of AMH and FSH values with LBR was assessed by multivariate logistic regression in different maternal age populations, separately.

Main results and the role of chance: LBR differed among AMH/FSH groups in all three populations analyzed (p<0.003). In the total patient population, LBR were 24.2% (381/1573), 16.8% (40/238), 10.9% (66/606) and 11.4% (47/413), in groups A, B, C and D, respectively. When the analysis was restricted to patients > 35 years old, LBR were 17.8% (164/923), 16.0% (25/156), 8.2% (40/489) and 9.9% (33/334), respectively. In patients > 35 years old, when discordant values of AMH and FSH were present (groups B and C), a markedly higher LBR was observed in the group presenting the prognostically favorable AMH value (AMH>1, group B). Conversely, in women ≤ 35 years old, a higher LBR was observed in the group presenting the prognostically favorable FSH value (FSH≤10, group C); LBR were 33.1% (215/650), 18.3% (15/82), 22.2% (26/117) and 17.7% (14/79), respectively. Multivariate logistic analysis revealed that AMH, but not FSH, is correlated with LBR, both overall [AMH: 2.3 OR, (1.8-2.9) 95% IC, p<0.0001; FSH: 0.8 OR, (0.6-1.0) 95% IC; p=0.076] and in women > 35 years [AMH: 2.2 (1.6-3.0), p<0.0001; FSH: 1.0 (0.7-1.4), p=0.86]. In contrast, in women ≤ 35 years, FSH was more significantly correlated with LBR than AMH [AMH: 1.5 (1.0-2.3), p=0.036; FSH: 0.5 (0.4-0.9), p=0.008].

Limitations, reasons for caution: Our study is subjected to the intrinsic limitations of a retrospective analysis, the results presented could have been affected by variables that are uncontrolled for.
Wider implications of the findings: Our results suggest the possibility of developing novel prognostic strategies in ICSI/IVF practice, utilizing FSH basal levels as predictors of live birth for women younger than 35 years, and AMH levels for older patients. In addition, our findings may help in counselling patients and managing their expectations.
Trial registration number: Not applicable

P-693 Adiponectin mediates apoptotic responses in endometrial carcinoma in PCOS patients by the AMPK pathway.

S. Chen[1], X. Chen[1]

[1]Sun Yat Sen Memorial Hospital- Sun Yat Sen University, reproductive endocrinology, guangzhou, China

Study question: we hypothesize that adiponectin regulates target genes expression CYP19 through AMPK pathway, and thereby plays an important role in the tumorigenesis and tumor-development in PCOS patients.
Summary answer: We elucidate the mechanism of APN-mediated estrogen-dependent tumorigenesis and development of endometrial cancer in PCOS patients ,reveal new intervention targets for its prevention and treatment.
What is known already: Estrogen-dependent endometrial cancer is one of the most fatal long-term complications of polycystic ovary syndrome (PCOS). Our previous research confirmed that compared with age-matching health women, PCOS and endometrial cancer patients had lower adiponectin (APN) level, which showed a negative correlation with increasing expression levels of CYP19 and ERα in tumors. Our previous studies indicated that APN stimulated AMPK phosphorylation, reducing the expression of CYP19 in cancer cells, and sequentially inhibiting cancer cell proliferation and invasion. Furthermore, inhibiting AMPK pathway could block the above effect of APN.
Study design, size, duration: Human endometrial cancer cell lines Ishikawa and KLE underwent treatment with APN at various concentrations for different durations, followed by assessment of cell proliferation by methyl thiazolyl tetrazolium (MTT) assay. Real-time quantitative PCR and western blot analysis were used .Bioinformatics analysis, siRNA interference and CHIPS technology were used to detect the potential transcription factors involved in APN-stimulated AMPK pathway.
Participants/materials, setting, methods: cell culture(Human endometrial cancer cell lines Ishikawa and KLE) .Methods and endpoints used cell numbers/proliferation, Westerns, quantitative PCR, bioinformatics analysis, siRNA interference and CHIPS technology.
Main results and the role of chance: At different time points of APN treatment at different concentrations, showed by MTT assay, the proliferation rates of Ishikawa and KLE cell lines were inhibited in a dose-dependent and time-dependent manner between APN groups and the control group($P<0.5$). QT-PCR analyses showed that with the increasing concentrations of APN(0,10,30ug/ml), stimulating IK and KLE cells for 24 hours, the mRNA expression of CYP19 was markedly dose-dependent down-regulated, and the mRNA expressions of AdipoR1 and AdipoR2 were significantly increased ,compared with the control group (0ug/ml,all $P<0.05$). Western blot analysis revealed the time-dependent decreasing protein expression of the potential transcription factors CEBPA selected by bioinformatics analysis,stimulating ECC-1 at 30ug/ml for 0,5,15,30 min. RT-PCR and western blot analysis showed that the mRNA and protein expressions of CYP19 was dreseasing when detecting the ECC-1 transfected with siCEBPA for 48h($P<0.05$). Furthermore,CHIPS technology reveal there is direct combination of CEBPA and CYP19 promoter sequences.
Limitations, reasons for caution: only in vitro, cell culture.
Wider implications of the findings: reveal new intervention targets for its prevention and treatment
Trial registration number: 8140060623

P-694 Follitropin delta – first human cell line derived recombinant FSH for AMH and body weight adjusted ovarian stimulation – a real world data analysis

A. Bachmann[1], S. Kissler[2], I. Laubert[3], P. Mehrle[4], A. Mempel[5], C. Reißmann[6], D. Sauer[7], S. Tauchert[8], A.P. Bielfeld[9]

[1]University Hospital Frankfurt, Reproductive Endocrinology- ReProGyn, Frankfurt, Germany ;
[2]Center for Reproductive Medicine and Endocrinology Frankfurt, Center for Reproductive Medicine and Endocrinology Frankfurt- Germany, Frankfurt, Germany ;
[3]Fertility Clinic Munich, Fertility Clinic Munich- Germany, Munich, Germany ;
[4]Reproduktionsmedizin München, Practice center for reproductive medicine Munich, Munic, Germany ;
[5]Center for Reproductive Medicine at the Promenade- Muenster- Germany, Center for Reproductive Medicine at the Promenade- Muenster, Muenster, Germany ;
[6]Profertilita center for reproductive medicine- Regensburg, Profertilita center for reproductive medicine- Regensburg, Regensburg, Germany ;
[7]Center for Reproductive Meicine and Endocrinology, Center for Reproductive Meicine and Endocrinology, Frankfurt, Germany ;
[8]IVF-Saar- Center for Reproductive Medicine and Gynecological Endocrinology, IVF-Saar- Center for Reproductive Medicine and Gynecological Endocrinology, Saarbrücken, Germany ;
[9]Duesseldorf University Hospital, Department of OB/GYN and REI- UniKiD, Duesseldorf, Germany

Study question: Does the first recombinant FSH with a dosing algorithm affirm efficacy data observed in pivotal trials in a real world setting?
Summary answer: A good clinical pregnancy rate and mean number of oocytes was obtained so that Follitropin delta appears as an efficient agent for daily practice.
What is known already: Follitropin delta (REKOVELLE®, Ferring Pharmaceuticals) is the first recombinant FSH (rFSH) derived from a human cell line with a dosing algorithm based on body weight and AMH. Due to different glycosylation profiles, it has a lower clearance compared to other rFSH preparations and therefore induces a higher ovarian response when administered in equal doses of biological activity. Non-inferiority of clinical pregnancy and implantation rate for controlled ovarian stimulation with conventional rFSH treatment regime could be demonstrated in the multicenter randomized pivotal trial ESTHER-1, but there is an increasing demand for daily practice data with non-selected patients.
Study design, size, duration: In a multi-center analysis comprising eight centers for reproductive medicine in Germany, observational data of 360 women who underwent ovarian stimulation with Follitropin delta have been evaluated as part of the quality control from January 2018 to June 2019. The data were analyzed retrospectively.
Participants/materials, setting, methods: Mean age was 33.53 (± 3.83) years. AMH levels ranged from 2.5% < 0.5 ng/ml to 18.6% > 5.6 ng/ml with 79.4% of all AMH measurements above 2.03 ng/ml. Patients were included when the following prerequisites were met: data on ovulation triggering available, less than six previous cycles, no additional gonadotropin medication during stimulation with Follitropin delta. No selection regarding age, serum AMH level or BMI. The data were collected retrospectively and anonymized before analysis.
Main results and the role of chance: Based on the above criteria, a total of 360 women were included in the data analysis. Downregulation was performed in 96.6% of the first Follitropin cycle in the GnRH antagonist protocol, in 3.4% in the long GnRH agonist protocol. The mean daily dose was 9.03µg with a mean total dose of 89.50µg in a mean stimulation time of 9.9 days. In the AMH ranges between 2.031 and 3.5 ng/ml 8 - 14 oocytes were obtained in 44.1% of the patients whereas in the AMH range between 3.51 and 5.6 this proportion was even 50.4%. The mean number of oocytes obtained in the first Follitropin delta cycle was therefore 11.2 (± 6.7) oocytes with 42.1% of patients having between 8 and 14 oocytes. The average clinical pregnancy rate in the first fresh cycle was 38.2% with a mean of 1.4 embryos per transfer. The cumulative pregnancy rate though was 49.4% for the first stimulation cycle including cryo cycles. Due to the descriptive character of the analysis no adjusting for multiple testing was applied.
Limitations, reasons for caution: Shortcomings of the analysis might be a selection bias of the centers. According to the daily practice setting there were no present uniform criteria on how many embryos were transferred.
Wider implications of the findings: The goal of obtaining an adequate number of oocytes (8-14) using the Follitropin delta dosing algorithm was reached in 42.1% of patients despite a wide range of AMH values, while achieving good clinical pregnancy rates. Hence, algorithm based controlled ovarian stimulation with Follitropin delta is effective in daily practice.

Trial registration number: not applicable

P-695 Individualised follitropin delta dosing for ovarian stimulation in daily practice in a Dutch IVF centre.

P. Wijngaard-Boom[1], E. Creutzberg[2], T. Stijnen[3], J.S.E. Laven[1]

[1]Erasmus University Medical Centre, Reproductive Medicine, Rotterdam, The Netherlands ;
[2]Ferring BV, Gynaecology, Hoofddorp, The Netherlands ;
[3]Leiden University Medical Centre, Biostatistics, Leiden, The Netherlands

Study question: This study investigates in a real life setting the use of follitropin delta with individualised dosing regimen in patients undergoing IVF treatment.
Summary answer: The use of follitropin delta, in real-world clinical setting with a broad and varied patient population, shows results aligned with the ESTHER-1 registration trial (NCT01956110).
What is known already: Individualised follitropin delta dosing based on anti-Müllerian Hormone (AMH) and body weight (BW) is as effective as treatment with Chinese hamster ovary (CHO) derived follitropin alfa, and leads to a more predictable ovarian response within the target of 8-14 oocytes and improved safety. Results of the ESTHER-1 trial show: similar pregnancy rates; less frequent excessive ovarian response and measures to prevent ovarian hyperstimulation syndrome (OHSS); more women with a target ovarian response (8–14 oocytes); fewer poor responses with AMH<15 pmol/L; fewer excessive responses with AMH≥15 pmol/L.
Study design, size, duration: Descriptive analysis from real-world data obtained in the Erasmus University Medical Centre. Since its introduction in 2017, 665 patients have been treated with follitropin delta in our centre up to and including September 2019. 444 of these patients had a regular ovulatory menstrual cycle and were treated with follitropin delta in a GnRH-antagonist protocol, with the dose determined by the approved dosing algorithm, based on patient's BW and AMH.
Participants/materials, setting, methods: Women are prescribed follitropin delta according to the approved dose algorithm based on the patient's BW and serum AMH levels as measured by the automated Elecsys® AMH plus immunoassay (Roche Diagnostics International). Results of the treatment were collected from the clinical records. The outcomes of the first IVF cycle including frozen embryo transfers are described. Data were described as mean (SD), median (interquartile range) or proportions and statistically analyzed using t-test and chi-square test.
Main results and the role of chance: In the 444 patients, almost all patient characteristics were significantly different compared to those of the patients in the follitropin delta arm of the ESTHER-1 trial, except age: 33.4 (4.6) and 33.4 (3.9) years, NS. Mean (SD) BW was 69.9 (13.1) and 64.7 (10.7) kg (p<0.00001), primary subfertility diagnosed in 54.3% and 70.7% of the patients (p<0.00001), and proportion of patients naive to IVF was 65.5% and 100% respectively (p<0.00001). Median AMH (interquartile range) was also significantly different: 15 (10-24.3) and 16.3 (9-24.8) pmol/l (p=0.04).

Retrieved number of oocytes was 10.2 (5.6) and 10.0 (5.6), 8-14 oocytes were retrieved in 46.6% and 43.3% of patients and the number of cleavage stage embryos was 5.6 (5.0) and 5.4 (3.7) (all not significantly different). Ongoing pregnancy rate per started cycle was 24.5 and 30.7% (p=0.04). Cumulative pregnancy rate including frozen embryo transfers was 37.3% (no data from ESTHER-1 trial). More cycles were cancelled with an insufficient number of follicles: 10.8% and 3.8% (p<0.00001) and more preventive interventions for OHSS (GnRH-agonist trigger and/or freeze all) were performed: 5.4% and 2.3% (p=0.005). Severe OHSS requiring hospitalization was reported for 3 and 2 patients.
Limitations, reasons for caution: This is an analysis of the use of follitropin delta in patients in daily clinical practice. A heterogeneous population with different phenotypes was treated, which was different from the patients treated in the clinical trials performed earlier.
Wider implications of the findings: Individualised follitropin delta treatment of a broad patient population as presented in the real-world clinical setting shows that this new dosing regimen is able to predict ovarian response in a similar way as shown in the results of a previously performed clinical trial (ESTHER-1), even though patient characteristics were different.
Trial registration number: not applicable

P-696 Addition of LH to a COS antagonist protocol is not associated with number of blastocysts or their morphology.

J.E. Schwarze[1], J. Crosby[1], G. Cavada[2]

[1]Reproductive Medicine Unit, OB/GYN Clinica Las Condes, Santiago, Chile ;
[2]Universidad de Chile, School of Public Health, Santiago, Chile

Study question: Is the addition of LH (either as hCG or rLH) to an antagonist COS protocol associated with the number and morphology of blastocysts.
Summary answer: Addition of LH is not associated with number of expanded blastocysts, nor an increase in good morphology.
What is known already: There is contradictory evidence that IVF/ICSI outcomes are improved by LH (either as hCG or rLH) supplementation during COS. Several outcomes have been analyzed with conflicting results: number of oocytes, pregnancy, abortion and delivery rates. One outcome that has not been evaluated is the morphology of blastocyst.
Study design, size, duration: We performed a hierarchical multiple to determine the impact of three different COS antagonist protocols on blastocyst morphology (expanded, cavitated, initial): rFSH (reference), rFSH/hMG and rFSH/rLH, . Confusion was accounted for by correcting for the following variables; age of female partner, number of oocytes retrieved, sperm source (donor, testicular and fresh ejaculate). The outcomes analyzed were number of expanded blastocysts, odd of having an expanded blastocyst, and odd of a blastocyst being expanded over cavitated/initial.
Participants/materials, setting, methods: 2,112 autologous IVF/ICSI cycles were performed in the Reproductive Medicine Unit at Clinica Las Condes between 2015 and 2019**.** Blastocyst morphology was classified as expanded, cavitated or initial. Multilevel regression analysis was performed to determine number of expanded blastocysts, odd of having an expanded blastocyst, odd of having an expanded blastocyst over initial blastocyst, respectively.
Main results and the role of chance: The mean age of female partner was 36 years (mode 37, SD 3.8). 20% of patients were administered rFSH, 48% hMG/rFSH and 32% rFSH/rLH. Blastocyst rate formation was 38%, 35% and 33%, respectively (Chi square, p=0.444).
Compared to rFSH alone, the addition of either hMG or rLH to an antagonist COS protocol was not associated with:

a) an increase in the number of expanded blastocysts (multilevel Poisson regression, rFSH alone (reference), hMG/rFSH IRR 1.08 (95% CI 0.93-1.26, p=0.301); rFSH/rLH IRR 0.86 (95% CI 0.73-1.92, p=0.092));
b) number of expanded blastocysts (multilevel mixed logistic regression, rFSH alone (reference), hMG/rFSH OR 1.09 (95% CI 0,76-1.40, p=0.847); rFSH/rLH OR 1.03 (95% 0,.74-5,60, p=0.162);
c) odd of a blastocyst being expanded versus cavitated/initial (rFSH alone (reference), hMG/rFSH OR 1.11 (95% CI 0.87-2.25, p=0.281); rFSH/rLH OR 1.02 (95% CI 0.93-2.00, p=0.839).

Limitations, reasons for caution: This was an observational study so there may be unknown variables that were not accounted for, as would have been in an RCT.
Wider implications of the findings: The addition of LH to a COS does not improve neither the number of blastocysts, nor the morphology of blastocysts. Therefore, the choice of hormones for COS should depend on factors like price and accessibility.
Trial registration number: NA

P-697 The individualization of the gonadotrophin starting dose in IVF/ICSI prevents OHSS in the same manner as the freeze-all strategy: a systematic review of the evidence

A.A. Marino[1], S. Gullo[2], A. Volpes[1], F. Sammartano[1], P. Scaglione[1], A. Allegra[1]

[1]ANDROS Day Surgery Clinic, Reproductive Medicine Unit, Palermo, Italy ;
[2]University of Palermo, Department of Psychology- Educational Science and Human Movement- Statistics Unit, Palermo, Italy

Study question: Is the individualization of the starting dose of gonadotrophins equivalent to the freeze-all strategy for preventing ovarian hyperstimulation syndrome (OHSS)?

Summary answer: The personalization of the gonadotrophin starting dose, in normal responders, is a valid strategy for assuring treatment safety similarly to the freeze-all policy.

What is known already: Nowadays, the freeze-all policy has gained popularity because it minimizes OHSS.

This increase was possible thanks to vitrification procedures. Consequently, "wild" ovarian stimulation seems to replace the starting dose personalization, based on female age, ovarian reserve markers, BMI, etc. Nevertheless, the freeze-all policy does not completely eliminate OHSS. Furthermore, not all clinics have adequate skills in vitrification procedures and patients may be displeased with a longer time to pregnancy

Another approach for minimizing OHSS is the tailoring of the starting gonadotrophin dose. No systematic review comparing these two different strategies, in terms of reduction of OHSS, has been yet performed.

Study design, size, duration: A systematic review, based on PubMed, Cochrane CENTRAL, EMBASE, was conducted to estimate and compare the effectiveness of the individualization of the starting dose and freeze-all strategy in the prevention of OHSS in normal responders. Following *PICOS*, inclusion criteria were: *Population*, normal responders; *Intervention*, individualization of the starting gonadotrophin dose OR freeze-all strategy; *Comparison*, no individualization OR fresh embryo transfer; *Outcome*, OHSS (primary one), clinical/ongoing pregnancy and live birth rates (secondary ones). *Study design*: RCT.

Participants/materials, setting, methods: Electronic and manual search, conducted from 1990 to 2019, yielded 184 studies. Two researches reviewed the studies independently, excluding 162 studies after the first screening and 12 studies after the second screening. Thus, 10 studies were included. The Mantel-Haenszel method was used for odds ratios (OR) and heterogeneity among studies (I^2). The results were expressed in terms of prevalence, OR, and 95% confidence intervals (CI). Standardized test and Cohen's *h* were used to compare outcomes.

Main results and the role of chance: Three studies with 2,293 participants for the freeze-all strategy (Vuong et al., 2018, Shi et al., 2018, Wei et al., 2019) and seven studies with 1,705 participants for individualized strategy (Popovic-Todorovic et al., 2003, Arce et al., 2014, Olivennes et al., 2015, Allegra et al., 2017, Nyboe-Andersen et al., 2017, Bosch et al., 2019, Peterson et al., 2019) were included in the review. None of these studies directly compared the two strategies. Preliminary analyses excluded interaction between covariates, defining intervention/control groups and outcome. In the individualization strategy, the analyses also considered cycles suspended for OHSS risk.

OHSS was significantly lower in women receiving individualized treatment [50/1,705] compared with controls [91/1,464] (OR=0.505, 95%CI 0.35-0.73, p=0.01; I^2=0%). Similarly, OHSS was significantly lower in freeze-all strategy group [14/2,293] compared with controls [35/2,296] (OR=0.403, 95%CI 0.22-0.76, p=0.01; I^2=0%). Indirect comparisons between the two strategies were carried out by calculating effect size (Cohen's *h*) on prevalence and by computing a z-test on the difference between natural log OR. The difference between the two strategies revealed a small effect size (Cohen's *h*=0.19) and yielded a non-significant z-value of 0.549 (p=0.29) suggesting that individualization of the starting dose is equivalent to the freeze-all strategy for preventing OHSS.

Limitations, reasons for caution: Comparison of the studies was indirect because no published study directly compared the freeze-all strategy with the individualization of the starting dose. In the selected studies, the definition of normal responders was not exactly the same but similar.

Randomized controlled trials comparing the two different strategies are necessary.

Wider implications of the findings: This systematic review indicates that, in normal responders, the individualization of the starting gonadotrophin dose reduces OHSS similarly to the freeze-all strategy. We believe that, albeit in the era of the freeze-all policy, the personalization of the starting gonadotrophin dose should be considered the first line of an IVF program.

Trial registration number: Not applicable

P-698 Cumulative live birth rate of poor ovarian response women won't increase after 4 complete IVF cycles

M. WANG[1], J. Guo[1], C. Fang[1], X. Liang[1]

[1]Sixth Affiliated Hospital of Sun Yat-sen University, Reproductive Medicine Center, Guangzhou, China

Study question: How does the cumulative live birth rate (CLBR) of poor ovarian response (POR) women change as oocyte retrieval cycles increase?

Summary answer: Although initiating more cycles increases the cumulative clinical pregnancy rate (CCPR) of POR women, the CLBR won't increase after 4 complete cycles.

What is known already: Women with POR went through repeated IVF/ICSI failures. Many oocyte retrieval cycles may be initiated to pursue additional success rate, but seldom can they get a live birth. Repeated trying on new oocyte retrieval cycles without promising prognosis may be a heavy burden for patients both financially and mentally.

Study design, size, duration: A retrospective cohort study of 2775 complete cycles of 1825 POR women according to the Bologna criteria were recruited at the Reproductive Medicine Center of the sixth affiliated hospital of Sun Yat-sen University from 2014 to 2018. A complete cycle defined as all fresh and frozen-thawed embryos derived from one ovarian stimulation were transferred.

Participants/materials, setting, methods: Infertile POR women who underwent their first IVF/ICSI treatment at the Reproductive Medicine Center were recruited. The optimistic and conservative estimates of CLBR and CCPR were calculated according the oocyte retrieval cycles. Cycles that had not achieved live birth but with embryos left were excluded. Cases involving oocyte thawing and donation were eliminated.

Main results and the role of chance: Basic characteristic: The mean age was 39.43 (SD=4.723) years old. 29.4% were primary infertility. The median duration of infertility was 4 (2, 7) years. The median basal serum FSH were 9.11 (7.01, 11.68) IU/L, basal AMH 0.62 (0.37, 0.93) ng/ml, and bilateral antral follicle count 4 (3, 6). 77.5% underwent IVF and the rest adopted ICSI. The proportions of ovarian stimulation protocols: mild stimulation protocol, 29.01%; Shanghai protocol, 27.6%; Antagonist protocol, 24%; long protocol 1.95%; natural cycle protocol 3.57% and others 6.92%. The median number of oocytes retrieved were 3 (2, 4).

The optimistic and conservative estimates of CCPR increased along with new cycles in the first 6 cycles (the optimistic estimates of the 1st to the 7th cycle: 25.48%, 42.38%, 59.02%, 69.78%, 73.71%, 84.19%, 84.19%; the conservative estimates of the 1st to the 7th cycle: 25.48%, 33.75%, 36.60%, 37.09%, 37.15%, 37.21%, 37.21%). But the optimistic and conservative estimates of CLBR rose as the cycles added in the first 4 cycles(the optimistic estimates of the 1st to the 7th cycle: 14.79%, 24.13%, 32.37%, 39.92%, 39.92%, 39.92%, 39.92%; the conservative estimates of the 1st to the 7th cycle: 14.79%, 18.79%, 19.95%, 20.22%, 20.22%, 20.22%, 20.22%), reached 39.99% and 20.22% respectively.

Limitations, reasons for caution: As a retrospective study, there were many cycles that had not achieved live birth but with embryos left were excluded. Thus, there were not enough cases in the 6th and 7th cycle, which may lead to deviation of the real condition.

Wider implications of the findings: It's not recommended to suggest more than 4 complete cycles for the POR women because the CLBR won't increase any more.

Trial registration number: not applicable

P-699 The use of follitropin delta for ovarian stimulation for in vitro fertilization in patients with anovulatory PCOS: results in clinical practice

J. Laven[1], P. Wijngaard-Boom[1], T. Stijnen[2], E. Creutzberg[3]

[1]Erasmus Medical Center, Reproductive Medicine, Rotterdam, The Netherlands ; [2]Leiden University Medical Centre, Biostatistics, Leiden, The Netherlands ; [3]Ferring BV, Gynaecology, Hoofddorp, The Netherlands

Study question: This study investigates in a real life setting the use of follitropin delta in patients with anovulatory Polycystic Ovary Syndrome (PCOS).

Summary answer: In patients with anovulatory PCOS, the use of follitropin delta according to the dose algorithm results in good clinical results with a favorable safety profile.

What is known already: Individualized follitropin delta dosing based on Anti-Müllerian hormone (AMH) and body weight (BW) for IVF treatment is as effective as follitropin alfa, and leads to a more predictable ovarian response within the target of 8-14 oocytes and improved safety. In pivotal trials performed with follitropin delta, only women with a regular menstrual cycle (24-35 days) were included. In our clinic, up to 40% of IVF cycles were canceled because of

hypo- or hyperresponse if patients with anovulatory PCOS were treated with other traditional stimulation protocols.
Study design, size, duration: Since its introduction in The Netherlands in 2017, 665 patients have been treated with follitropin delta in the Erasmus University Medical Centre up to and including September 2019. In total 74 of these patients had been diagnosed with PCOS prior their treatment. The real world data of their first IVF cycle including frozen embryo transfers are described.
Participants/materials, setting, methods: All patients with PCOS have been diagnosed by the Rotterdam criteria (at least 2 out of 3 features: oligo- or amenorrhea, polycystic ovarian morphology, hyperandrogenism). AMH was measured using the automated Elecsys® AMH plus immunoassay (Roche Diagnostics International) and the dose of follitropin delta was determined using its approved dosing algorithm, based on patient's BW at start of treatment and AMH. Results of the treatment were collected from the clinical records.
Main results and the role of chance: Patients with PCOS had a mean (SD) age of 31.5 (4.0) years, body weight 69.5 (10.2) kg and AMH 40.7 (22.5) pmol/L. The mean duration of subfertility was 35.8 (28.6) months. 54.1% had primary subfertility and 40.5% of patients had received IVF treatment(s) prior to the treatment cycle with follitropin delta. The mean dose of follitropin delta used was 8.4 (2.1) µg/day. The mean number of follicles ≥11mm at day of last ultrasound was 14.3 (8.5). The mean number of oocytes retrieved was 12.1 (7.0) and the mean number of cleavage stage embryos 6.9 (4.6). Target response (8-14 oocytes) was reached in 29.2% of patients; 33.8% had <8 oocytes and 36.9% >14 oocytes. Per started cycle, 44.6 % had a positive urine human chorionic gonadotropin test after the first fresh transfer and 18.9% after cryotransfer (following unsuccessful fresh transfer or cryotransfer after freeze all). Overall cumulative ongoing pregnancy rate (≥ 11 weeks) per started cycle was 39.2%. The cycle was cancelled in 12.2% of the patients (5.4% due to hyperresponse and 6.8% due to insufficient number of follicles). 21.6 % needed preventive interventions for OHSS (Gonadotropin-releasing hormone-agonist trigger and/or freeze all); no cases of severe OHSS requiring hospitalization occurred.
Limitations, reasons for caution: This is a descriptive analysis of the use of follitropin delta in PCOS patients. A heterogeneous population with different PCOS phenotypes was treated, with no control group to compare them to.
Wider implications of the findings: Clinical results as collected in routine clinical practice are promising, showing a favorable effectivity-safety profile. To conclude whether follitropin delta applied in accordance with its dosing algorithm can be used safely in PCOS patients without compromising its effectivity, further comparative investigations with larger patient groups should be conducted.
Trial registration number: not applicable

P-700 Next generation sequencing results on 18 genes are not related to phenotype in a large cohort of patients with premature ovarian

S. Eskenazi[1], A. Bachelot[2], J. Hugon Rodin[3], G. Plu-Bureau[3], A. Gompel[3], S. Catteau-Jonard[4], D. Molina-Gomes[5], D. Dewailly[6], C. Dode[7], S. Christin-Maitre[8], P. Touraine[9]

[1]Saint Antoine Hospital, Ile de France, Paris, France ;
[2]Pitié-Salpètrière Hospital, Ile de France, Paris, France ;
[3]Port-Royal Hospital, Ile de France, Paris, France ;
[4]CHU de Lille, Nord Pas de Calais, Lille, France ;
[5]Poissy/Saint-Germain-en-laye Hospital, Ile de France, Paris, France ;
[6]CHU Lille, Nord Pas de Calais, Lille, France ;
[7]Cochin Hospital, Ile de France, Paris, France ;
[8]Saint-Antoine Hospital, Ile de France, Paris, France ;
[9]Pitié-Salptrière Hospital, Ile de France, Paris, France

Study question: What is the prevalence of gene variants in a large prospective multicentric cohort of patients with premature ovarian insufficiency (POI) and is phenotype correlated to genotype?
Summary answer: A high percentage of patients presented gene variants detected by NGS analysis (38%) and phenotype was not correlated to genotype.
What is known already: Premature ovarian insufficiency is defined by loss of ovarian activity before the age of 40, and is characterized by menstrual disturbance (amenorrhea or oligomenorrhea) with raised gonadotropins and low estradiol. POI remains idiopathic in more than 70% of cases. However, over the past years, many candidate genes have been identified. Nevertheless their prevalence and pathogenicity are still difficult to establish.
Study design, size, duration: Cohort study on 269 patients from 2013 to 2017
Participants/materials, setting, methods: Two hundred and sixty-nine well-phenotyped POI patients were screened for variants in 18 known POI genes *(BMP15, DMC1, EIF2S2, FIGLA, FOXL2, FSHR, GDF9, GPR3, HFM1, LHX8, MSH5, NOBOX, NR5A1, PGRMC1, STAG3, XPNPEP2, BHLB and FSHB)* using next generation sequencing (NGS). Abnormalities were classified as "variant" or "variant of unknown signification" (VUS) according to functional tests if any, otherwise according to several algorithms (SIFT, Polyphen-2, mutation taster).
Main results and the role of chance: One hundred and two patients (38%) were identified with at least 1 genetic abnormality on 1 to 5 genes. Seventy-two patients (25%) presented at least 1 variant. Fifty patients presented at least 1 uncommon variant of unknown significance (VUS)(17%). Thirteen patients (4.5%) had combined abnormalities (variant and VUS). *NOBOX* variants were the most frequent autosomal gene variant implicated in POI (9% of the patients). Interestingly, previous familial history of POI, ethnicity, age at POI, primary amenorrhea or secondary menstrual disturbance occurrence was not significantly different between the different genotypes.
Limitations, reasons for caution: We possibly analyzed altogether genes with different pathogenicities, which may have influenced our results. Assessing pathogenicity for each genetic abnormality remains challenging. Moreover, it has been shown that for a single gene, different types of variants may result in variable phenotype. Non-genetic factors may also be involved in POI development.
Wider implications of the findings: NGS analysis should be proposed to every POI patient to progress in understanding gene pathogenicity. Finding a variant may help patients to accept the diagnosis of POI and improve their compliance to treatment. It can also be relevant for female relatives for whom fertility preservation could be proposed
Trial registration number: not applicable

P-701 In vitro maturation of oocytes as an (the only?) option for patients with FSH resistance or oocyte maturation failure: a case series.

F. KRIEF[1], I. Cedrin Durnerin[2], S. Sarandi[3], B. Dagher Hayeck[2], M. Peigné[2], C. Sifer[3], M. Grynberg[4]

[1]Hôpital Jean Verdier, Médecine de la Reproduction et Préservation de la Fertilité, Bondy, France ;
[2]Hôpital Jean Verdier, Service de Médecine de la Reproduction et Préservation de la Fertilité, Bondy, France ;
[3]Hôpital Jean Verdier, Biologie de la Reproduction, Bondy, France ;
[4]Hôpital Béclère, Médecine de la Reproduction et Préservation de la Fertilité, Clamart, France

Study question: How viable is *in vitro* maturation (IVM) of oocytes in patients with FSH resistance or oocyte maturation failure following ovarian stimulation?
Summary answer: IVM of oocytes provides acceptable chances of live births in patients with FSH resistance or oocyte maturation failure following ovarian stimulation.
What is known already: The concept of IVM relies on the capacity of immature oocytes to resume meiosis *in vitro* upon release from their antral follicular environment. Some patients present ovaries that are not responsive to FSH or show recurrent oocyte maturation failure after controlled ovarian stimulation. For those patients oocyte donation remains the only viable option.
Study design, size, duration: This study is a retrospective case series showing our experience with IVM for patients with resistant ovary syndrome and oocyte maturation failure from 2015 to 2019. The diagnosis of resistant ovary syndrome was considered in patients showing normal antral follicle count and serum anti-Müllerian hormone levels despite high FSH levels and absence of response to exogenous FSH administration. Oocyte maturation failure was considered when no mature oocyte was obtained after at least 2 ovarian stimulation.
Participants/materials, setting, methods: Eleven patients were included. Three patients had oocyte maturation deficiency while eight presented with ovarian resistance to FSH. All underwent at least one IVM cycle. Immature oocyte

retrieval was systematically performed 36 hours after hCG administration. After ICSI, all cleavage stage embryos were frozen and transferred during the subsequent cycle under hormonal replacement therapy.

Main results and the role of chance: Three patients with oocyte maturation deficiency, aged 34, 29 and 28 years underwent a total of 6 IVM cycles. Their antral follicle count and serum AMH (in ng/mL) levels were respectively 13 and 1.52 ; 23 and 7 ; 22 and not available.

The first 2 women had only cycle with 1 and 2 cumulus oocytes complexes recovered without metaphase 2 oocyte obtained following IVM. The third patient had 4 cycles with a mean of 8.5 immature oocytes retrieved. After a mean maturation rate of 64.7%, seven embryos were frozen, leading after the transfer of 2 embryos to one live birth.

Eight patients with resistant ovary syndrome underwent 20 IVM cycles. Mean antral follicle count and serum AMH levels were 23.7 and 5.1ng/mL respectively). An average of 23.7 ± 20 immature oocytes were collected, leading after a 40.5% maturation rate, to 30 embryos cleavage stage embryos. Five fresh embryo transfers and 12 frozen thawed embryo transfers resulted in 3 pregnancies and four live births of healthy babies. The live birth rate was 15% per IVM cycle and 37.5% per patient.

Limitations, reasons for caution: Due to the very low prevalence of oocyte maturation failure and ovarian resistance to FSH, we report results from a small population, which unable drawing strong conclusion. Further data on the use of IVM in these clinical situations are needed.

Wider implications of the findings: Patients with ovarian resistance to FSH probably represent the most interesting population for being offered IVM. Due to more uncertain results, considering IVM in oocyte maturation failure may be questionable.

Trial registration number: None

P-702 Ovarian Stimulation with rFSH+rLH vs. rFSH alone in poor/suboptimal/normal responders undergoing IVF: real life analysis of 1382 patients stratified for the number of retrieved oocytes

S. Canosa[1], N. Mercaldo[1], A.R. Carosso[1], F. Evangelista[1], C. Racca[1], A. Ruffa[1], G. Gennarelli[1], A. Revelli[1], C. Benedetto[1]

[1]Physiopathology of Reproduction and IVF Unit- S. Anna Hospital, Department of Surgical Sciences, TORINO, Italy

Study question: Is the LBR (Live Birth Rate) of expected poor/suboptimal/normal responders undergoing IVF comparable whether Controlled Ovarian Stimulation (COS) is performed using rFSH+rLH or rFSH alone?

Summary answer: In poor/suboptimal/normal responders, rFSH+rLH stimulation increases oocyte maturation rate vs. rFSH alone, and gets comparable LBR when patients with the same oocyte yield are considered.

What is known already: No large studies comparing rFSH+rLH with rFSH alone have been performed so far in IVF patients, and the usefulness of adding rLH to rFSH in this patients is still debated. Studies in unselected patients showed that rLH addition to rFSH was unable to improve IVF outcome, but some other studies showed increased success rates in some subsets of patients. A recent meta-analysis gathering a series of small studies suggested that rLH supplementation might result in a higher clinical pregnancy rate in the so-called poor responders.

Study design, size, duration: This large retrospective, real life study included 1382 expected normal/suboptimal/poor responders aged 20-43 years, with AMH≤2.5, AFC≤15, no diagnosis of polycystic ovary syndrome or history of severe ovarian hyperstimulation syndrome. These patients underwent IVF treatment at our IVF Unit between 2004 and 2019. Among them, 753 were stimulated with rFSH+rLH (rFSH+rLH group) and 629 with rFSH alone (rFSH alone group).

Participants/materials, setting, methods: The clinical characteristics and reproductive outcome of patients in the two groups were compared using t-test or chi-squared test, as appropriate. As the LBR is known to increase with the number of retrieved oocytes, a further sub-analysis was performed, in which the two different COS regimens were compared after stratification for the number of retrieved oocytes, considering patients with the same number of available oocytes.

Main results and the role of chance: The two patient groups showed significant differences in age, years of infertility, AMH, antral follicle count, that were in favour of rFSH alone, confirming how in real life, the rFSH+rLH regimen is often reserved to poor prognosis patients. Accordingly to these prognostic factors, the number of retrieved oocytes was significantly higher in rFSH alone group, but the proportion of mature, metaphase II oocytes was slightly higher in patients receiving rLH. Also the LBR/cycle (24% vs 19.5%; p<0.05) was significantly higher in patients treated with rFSH alone, having a better a priori prognosis.

Considering only the 293 poor responders according to Bologna criteria (250 of which received rFSH+rLH, 43 rFSH alone), we observed that despite having a significantly higher mean age (38.3±3.5 vs 36.2±4.4; p<0.01) patients who received rFSH+rLH produced a significantly higher proportion of mature oocytes (88.4±22.4 vs 74.6±27.6; p<0.01), and finally obtained comparable LBR/cycle (14% vs 14.4%).

The equivalence between rFSH+rLH and rFSH alone-treated patients was confirmed after stratifying patients for the number of retrieved oocytes. Although in these smaller subgroups the differences in basal characteristics were still observed (in favour of rFSH alone group), in all subgroups the LBR/cycle resulted comparable for the two regimens.

Limitations, reasons for caution: This real life, retrospective study compares two patient populations with marked differences in basal characteristics, with a selection bias in favour of rFSH-alone group. What observed should be considered as purely indicative of rLH addition effect, and should be verified in adequately powered and designed trials.

Wider implications of the findings: In poor/suboptimal/normal responders, rLH addition to rFSH results in a higher proportion of mature oocytes and in comparable LBR despite significantly worse prognostic factors. This could suggest a favorable effect of rLH on oocyte quality and/or endometrial receptivity, that should be further studied.

Trial registration number: Not applicable

P-703 No embryonic development after IVF: an analysis of the subsequent IVF cycle

A.I. Athanasiou[1], R. Setton[1], N. Shah[1], Z. Rosenwaks[1], S. Spandorfer[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine-, New York, U.S.A.

Study question: Are certain treatment modifications associated with improved embryonic development after a failed IVF cycle?

Summary answer: Following an IVF cycle with no embryonic development, longer stimulation and the use of ICSI in the subsequent cycle are associated with improved embryonic development.

What is known already: The failure of all embryos in an IVF cycle to develop normally is a challenge for clinicians. There are several factors that influence embryonic development during an IVF cycle, including the stimulation protocol, the timing of the final oocyte maturation trigger, oocyte quality, successful fertilization and embryo culture conditions. While common practice is to trigger later for poor maturation and earlier for poor embryo quality, there is little guidance for total failure or arrest of embryonic development.

Study design, size, duration: This is a retrospective cohort study in which all patients who underwent IVF at an academic medical center between 2013 and 2019 were screened for inclusion. Patients were included if they had an IVF cycle at our center that resulted in no normal embryonic development for use (index cycle) and then underwent a subsequent IVF cycle at our center.

Participants/materials, setting, methods: A total of 207 IVF cycles that resulted in no embryonic development and the subsequent cycles were included. Patients were stratified by those who had embryos suitable for use in the subsequent cycle (group A) and those who did not (group B). Comparison was made between the index cycle that had no embryos and its subsequent cycle using a t-test. Chi-square test was used to compare modifications in IVF parameters between groups A and B.

Main results and the role of chance: Compared to the index IVF cycle, group A had significant increases in: lead follicle size (19.7 vs. 20.5mm, p=0.0006), number of days past an E2 level of 400 pg/mL (3.93 vs. 4.48, p=0.0011) and a lead follicle size of 14 mm (3.28 vs. 3.61, p=0.01), days of stimulation (10.8 vs. 11.5, p=0.02), number of embryos on day 3 (2.5 vs. 4.3, p<0.0001) and average cell count per embryo (3.9 vs. 6.4, p<0.0001), peak E2 (1506 vs. 1790 pg/mL, p=0.008), and number of oocytes harvested (7.5 vs. 9.1, p=0.01), mature oocytes (5.3 vs. 7.1, p=0.0008), and normal fertilization (2.1 vs. 4.4, p<0.0001).

However, there were no significant differences in total gonadotropin use and the time from trigger to retrieval. Conversely, compared to their index IVF cycles, group B had no statistically significant differences for any of these parameters. The subsequent cycle of group A had a significant increase in the use of ICSI (96.8%) compared to group B (88.2%, p=0.002). The groups showed no statistically significant difference when comparing whether or not there was a change in protocol type, use of oocyte activation, change in starting dose, delayed stripping, use of autologous endometrial co-culture, and change or type of trigger used.

Limitations, reasons for caution: This study is limited by a relatively small sample size; thus, the impact of certain protocol modifications may have been underestimated. Furthermore, only the cycle immediately following the failed cycle was analyzed.

Wider implications of the findings: To our knowledge, this is the first study that compares IVF cycles with no embryonic development with their immediate subsequent cycles. We believe this study provides a better understanding of changes that can improve embryonic development after failed IVF cycles due to total embryonic arrest.

Trial registration number: N/A

P-704 Luteal Phase Stimulation May be Better Than Follicular Phase Stimulation in Patients with Diminished Ovarian Reserve

H.B. Zeyneloglu[1], Y.A. Tohma[2], G. Onalan[2]

[1]BASKENT UNIVERSITESI, Kadin Hastaliklari ve Dogum ABD, ANKARA, Turkey ;
[2]Baskent University, Kadin Hastaliklari ve Dogum ABD, Ankara, Turkey

Study question: Are the mean numbers of the total number of oocytes retrieved, mature MII oocytes,pronucleus,grade 1 embryos and frozen embryos after follicular phase and luteal phase stimulations (FPS and LPS) different?

Summary answer: More number of mature oocytes and frozen embryos in LPS were obtained than FPS in the group of patients with diminished ovarian reserve.

What is known already: Despite advances in assisted reproductive technologies in recent years, treatment of patients with diminished ovarian reserve is still difficult for clinicians. However, as our knowledge of the follicular development increases, this group is better understood and newer treatment modalities are being developed. Multiple follicular recruitment waves in the same menstrual cycle is the basis of this new treatment option experienced early from fertility preservation strategies including luteal phase stimulation (LPS) or dual stimulation. Although there are many studies regarding this topic, it is not clear whether (FPS) or LPS yields better outcomes in this patient group.

Study design, size, duration: This retrospective case control study was conducted with 18 patients with diminished ovarian reserve (Group 1) and 11 normoresponder infertile patients (Group 2) undergoing two ovarian stimulations (FPS and LPS) and two oocyte retrievals in two separate menstrual cycles in the Baskent University Infertility Clinic between April 2019 and December 2019.In our clinic, diagnosis of diminished ovarian reserve was made by AMH <1.2 ng/mL and a total AFC less than 5 detected with transvaginal ultrasound.

Participants/materials, setting, methods: In group 1, all embryos were frozen on day 3 or 5, whereas some patients in Group 2 had transferred embryos in FPS but did not get pregnant, their LPS embryos were frozen. Patients' data included controlled ovarian hyperstimulation performance such as peak E2 level, the total number of oocytes retrieved, mature MII oocytes, pronucleus, grade 1 embryos, good quality embryos and frozen embryos.

Main results and the role of chance: The mean age of the patients in Group 1 and 2 were 36.0 ± 4.8 and 33.4±6.0 years, respectively. In group 1, total retrieved oocyte and total MII oocyte numbers were significantly higher in LPS when compared to FPS; 4.19 vs. 2.2, (p: 0.005) and 2.67 vs. 1.72, (p:0.044), respectively. Peak E2 levels, pronucleus oocyte numbers were similar in both stimulation phases. Both Grade 1 embryo and frozen embryos numbers were higher in LPS cycle; 1.11 vs 0.55, (p:0.025) and 1.72 vs. 0.67, (p:0.009), respectively. Although Group 2 patients had better results in LPS regarding PN, grade 1 and good quality embryos, they did not reach statistical significance.

Limitations, reasons for caution: Main limitations of this study are retrospective design and small sample size. Therefore, well-designed, prospective, large-scale studies are required to improve our understanding of this topic.

Wider implications of the findings: We noticed better synchronization of follicles and recruitment of more better quality embryos in LPS. LPS may be beneficial especially in the patient with poor ovarian reserve with follicular asynchronization in the menstrual onset and in the patients who does not accept dual stimulation for psychological, economical or other reasons.

Trial registration number: Not applicable

P-705 Progesterone levels on day of ovulation trigger and ongoing pregnancy rates in fresh and frozen cycles: a secondary analysis of a randomised controlled multicenter trial

S. Stormlund[1], N. Sopa[2], A. Zedeler[1], J. Bogstad[3], L. Prætorius[1], H.S. Nielsen[3], A. Klajnbard[4], A.L. Mikkelsen[5], S. Ziebe[3], N. La Cours Freiesleben[1], C. Bergh[6], P. Humaidan[7], A. Nyboe Andersen[3], A. Pinborg[3], K. Løssl[3]

[1]Hvidovre University Hospital, The Fertility Clinic- Department of Obstetrics and Gynecology, Hvidovre, Denmark ;
[2]Hvidovre University Hospital, The Fertility Clinic- Department of Obstetrics and Gynecology, Hvidovre, Denmark ;
[3]University Hospital Rigshospitalet, Fertility Clinic, Copenhagen, Denmark ;
[4]Herlev University Hospital, The Fertility Clinic- Department of Obstetrics and Gynecology, Herlev, Denmark ;
[5]Holbæk University Hospital, The Fertility Clinic- Department of Obstetrics and Gynecology, Holbæk, Denmark ;
[6]Sahlgrenska University Hospital, Department of Obstetrics and Gynaecology, Gothenburg, Sweden ;
[7]Skive Regional Hospital, The Fertility Clinic, Skive, Denmark

Study question: What is the role of progesterone on trigger day in the decision between a freeze-all and fresh transfer strategy in IVF in regular cycling women?

Summary answer: In a clinical setting with strict trigger criteria measurement of progesterone on the day of trigger is not indicated.

What is known already: Premature progesterone elevation in the late follicular phase following controlled ovarian stimulation has been associated with decreased ongoing pregnancy and live birth rates in fresh ART cycles. The possible underlying mechanism is embryo-endometrial asynchrony leading to decreased endometrial receptivity and implantation failure. Few studies have investigated the reproductive outcomes in relation to progesterone levels on ovulation trigger day in fresh and frozen embryo transfers in a randomised controlled trial setting.

Study design, size, duration: Progesterone levels on trigger day was a secondary outcome in a multicenter, randomised trial including 460 women allocated 1:1 to either (1) GnRH agonist trigger and freeze-all strategy or (2) hCG trigger and fresh single blastocyst transfer. Recruitment was performed over a 2-year period. The aim was to investigate ongoing pregnancy rates within the two groups according to progesterone levels measured on ovulation trigger day.

Participants/materials, setting, methods: Women between 18 and 39 years with a regular menstrual cycle referred for their 1, 2 or 3rd ART treatment were included. Biobank samples were collected on trigger day in the ovarian stimulation cycle in both groups and were subsequently analysed for progesterone levels. Trigger criteria was defined as presence of three follicles ≥ 17 mm and all recruiting clinics adhered to this. Ongoing pregnancy was determined by transvaginal ultrasonography at gestational weeks 8 –10.

Main results and the role of chance: As treated analysis was applied including only those women who complied with allocated randomisation group and who reached blastocyst transfer. A total of 288 women were included in the analyses. In the present clinical setting with strict adherence to predefined trigger criteria the prevalence of progesterone elevation above 1.5 ng/ml was only 3.8% (11/288). Ongoing pregnancy was compared in women with progesterone levels below and above 1.5 ng/ml in both women undergoing fresh blastocyst transfer and women with postponed FET. In the fresh transfer group the pregnancy rate was 17% (1/6) when progesterone levels exceeded 1.5 ng/ml and 42% (55/131) when progesterone was below this cut-off level. This difference however, did not show statistical significance (p=0.4). In the freeze-all group, the ongoing pregnancy rates were 40% (2/3) when progesterone levels exceeded 1.5 ng/ml and 34% (49/146) below the cut-off level of 1.5 ng/ml (NS) indicating that progesterone levels on the trigger day in the fresh cycle do not impact results in subsequent FET cycles. Importantly however, the number of women with high progesterone levels in the present analysis was very low. Additional analyses

using different cut-off levels of high as well as low progesterone was attempted and showed similar results.
Limitations, reasons for caution: Sample size for the primary RCT was powered to detect at 13% increase in ongoing pregnancy between the two groups, however in the present secondary analysis only 288 of 460 randomised women were included. In addition, the number of women with progesterone levels above 1.5 ng/ml was very low.
Wider implications of the findings: In a clinical setting where final oocyte maturation is triggered as soon as three follicles ≥ 17 mm are present, the measurement of progesterone levels on trigger day seems of no real clinical relevance.
Trial registration number: Clinicaltrials.gov identifier: NCT02746562

P-706 Progestins vs gonadotropin releasing hormone analogues for pituitary suppression during ovarian stimulation for assisted reproductive technology, a systematic review and meta-analysis

S. Yildiz[1], P. Alexandru[1], S.G. Cekic[1], E. Turkgeldi[1], B. Ata[2]

[1]Koc University Hospital, Assisted Reproduction Unit, Istanbul, Turkey ;
[2]Koc University, Assisted Reproduction Unit, Istanbul, Turkey

Study question: Are progestins as effective as gonadotropin releasing hormone analogues for pituitary suppression in assisted reproduction ?
Summary answer: Progestins can effectively prevent premature ovulation in assisted reproductive technology (ART) cycles. However, the quality of evidence is yet low.
What is known already: GnRH antagonists have become the most commonly used agents for pituitary suppression during ovarian stimulation for over a decade. They require less injections, provide similar pregnancy rates and lower risk of ovarian hyperstimulation syndrome than the former standard of care, i.e. GnRH agonists. Progestins are also capable of suppressing endogenous luteinizing hormone (LH) secretion from the pituitary. However, early endometrial exposure to progestin preclude a fresh embryo transfer. Yet, with the advent of high-survival embryo vitrification and increasing number of oocyte cryopreservation cycles progestins are being more frequently used in ART.
Study design, size, duration: A systematic review and meta-analysis of comparative studies regardless of randomization. We searched electronic databases, trial registers, and websites from the date of inception until June 1, 2019. Seven studies involving a total of 1548 women were included.
Participants/materials, setting, methods: Three studies compared a progestin with a GnRH antagonist and four studies compared a progestin with a GnRH agonist.The primary outcome was live birth rate (LBR) per woman. Secondary outcomes were live birth or ongoing pregnancy (LB/OP) per woman and per embryo transfer (ET), ongoing pregnancy, clinical pregnancy, positive pregnancy test, numbers of oocytes and metaphase-two oocytes, duration of stimulation and gonadotropin consumption.
Main results and the role of chance: While progestins fared similar with GnRH antagonists regarding effectiveness and safety parameters, progestins were associated with significantly higher LB/OP per ET compared with the short GnRH agonist protocol (RR=1.49, 95% CI=1.16 to 1.91). Although progestin primed stimulation lasted significantly longer and required significantly more gonadotropins than the short GnRH agonist protocol, the differences were small and clinically negligible. Safety parameters were similar between progestins and GnRH agonists.
Limitations, reasons for caution: The presence of a limited number of trials/studies, most of which are not randomized nor accounts for every woman starting stimulation are drawbacks, preventing definitive conclusions on the subject.
Wider implications of the findings: If future high-quality trials confirm the findings of the present review, progestins can become the agent of choice for pituitary suppression in ovarian stimulation cycles when a fresh embryo transfer is not intended. This would be a real benefit by eliminating the need for relatively costly GnRH analogues.
Trial registration number: Not applicable

P-707 rLH supplementation during GnRh-antagonist delayed-start OPCs-syncronized IVF cycles run by Follitropin delta

A. Nazzaro[1], A. Salerno[2], M. De Martino[3], L. Di Iorio[2]

[1]Futuraivf - Reproductive Medicine Center, Human Reproduction Unit, Benevento, Italy ;
[2]Futuraivf-Reproductive Medicine Center, Human Reproduction Unit, Benevento, Italy ;
[3]Futuraivf-r Reproductive Medicine Center, Human Reproduction Unit, Benevento, Italy

Study question: May endocrine dynamics evaluation be used as a predictor of ovarian response and IVF outcome during IVF Follitropin delta driven cycles?
Summary answer: Endocrine dynamics identify women needing rLH supplementation during COS run by Follitropin delta and the threshold under which supplementation is mandatory
What is known already: Stimulation protocols, impact both oocyte quality and quantity via endocrine/paracrine signals, as oocyte quality is programmed well before ovulation. rLH supplementation increases pregnancy rate, in unselected patients, when it is supplemented as a routinary part of an IVF cycle accordingly to endocrine dynamics evaluations and during ovarian stimulation in poor responders or women of an advanced age.
Study design, size, duration: 200 unselected women were enrolled.A delayed-start protocol with individualized Follitropin delta dose,following daily 0,25 µg GnRh-antagonist administration was used.LH,E2, and Pg determinations were performed on day 3-4 of spontaneous menstruation (basal),at t0 (day of OCP stop),t1 (day of first GnRh-antagonist administration),t2 (first us scan),t3 (second us scan) to evaluate the need of rLH adding.An arbitrary cut off of 1,4 mIU/ml of circulating LH at any time was considered as the threshold for rLH tailored supplementation.
Participants/materials, setting, methods: 100 patients out of 200 (group I) received only tailored follitropin delta, whilst 100 patients (group II) received tailored rFSH plus rLH accordingly to endocrine dynamic evaluations.Primary outcomes were total number and MII retrieved oocytes,number of 2PN,top embryo quality rate.Secondary outcomes included total number of transferred embryos,BhcG/started cycle and BhcG/ET,implantation rate,clinical pregnancy rate/started cycle,-clinical pregnancy rate/ET.Data were analyzed by using the SPSS version 20.0: Mann–Whitney U-test and Z-test were used. Significance was set at $P<.05$
Main results and the role of chance: Serum E2 and P level at t2 and t3 did not show any statistical significant difference between the two groups.LH values at basal time (t0)were similar in both groups(5,60 + 2,70 vs 5,56 + 2,67 mIU/ml),whereas LH serological values at t2(2,95+3,54 vs 1,90+1,56 mIU/ml)and at t3 (2,70 + 2,15 vs 1,92+1,56 mIU/ml)were markedly reduced when COS was driven with Follitropin delta alone respect to the stimulation supplemented with rLH,reaching a statistically significant difference between the two groups (*p* value <.05 and <.002 respectively)at any time.Notably Metaphase II oocytes (6,32+3,22 vs 4,50+2,70),fertilization rate (4,18+2,16 vs 2,81+1,49;) and top scoring embryos (3,17+1,89 vs 2,16+1,25)showed a statistical significance difference in favour of women receiving and add back of rLH (*p* values <.00001;<.00001 and <.00001 respectively).Total dosage of Follitropin delta administration, total number of retrieved oocytes, total number of embryo transferred at day 2/3 were similar in the two groups.BhcG for started cycle and for embryo transfer,implantation rate and ongoing pregnancy rate for started cycle and for embryo transfer,whilst not reaching a statistically significant difference,showed a clear trend towards a better outcome in favour of the added rLH group(47,77% vs 37,96%; 50,91% vs 41,21%; 22,29% vs 16,4%; 38,50 vs 29,16%; 38,99 vs 29,6% respectively).
Limitations, reasons for caution: More robust studies are needed to have a clear comprehension of paracrine/endocrine signals regarding bidirectional cumulus-oocyte dialogue and to clarify who really needs of LH supplementation, which is the threshold under which supplementation with rLH is mandatory and, at what point of follicular stimulation we need to add rLH
Wider implications of the findings: rLH supplementation may increase pregnancy rate in Follitropin delta run IVF cycles when it is administered respecting the endocrine milieu throughout the entire length of follicular phase accordingly to endocrine dynamics. Other iatrogenic interference may further impair IVF outcome
Trial registration number: not applicable

P-708 Comparison of effects of Duphaston and Cetrotide on Oocyte and Embryo Quality in Women Undergoing Intracytoplasmic Sperm Injection

S. Jouhari[1], N. Motaref[2], A. Mohammadzadeh[2], N. Madadi[3], A. Ghoodjani[2]

[1]Avicenna Research Institute- ACECR, infertility, Tehran, Iran ;
[2]Avicenna Research Institute- ACECR, Reproductive Biotechnology Research Center, tehran, Iran ;
[3]Avicenna Research Institute- ACECR, Biotechnology Research Center, tehran, Iran

Study question: To compare the effects of Duphaston and Cetrotide on prevention of premature luteinizing hormone surge and characteristics of retrieved follicles and embryos in women undergoing intracytoplasmic sperm injection (ICSI).
Summary answer: Duphaston could be used as an appropriate medication instead of GnRH antagonists in women undergoing controlled ovarian hyperstimulation (COH).
What is known already: Premature luteinizing hormone (LH) surge is one of the causes of assisted reproductive technology (ART) cycle cancellation. GnRH antagonists are used as agents for the prevention of premature luteinizing hormone surge. It is needed to find novel approaches with improved efficacy and safety profile.
Study design, size, duration: This is a retrospective study, conducted in the Avicenna Infertility Clinic of Tehran, Iran. Women undergoing ICSI regimens for infertility treatment were recruited from September 2017 to December 2018. A total of 200 patients were assessed for eligibility, which was divided into two groups. Case group (100 patients) received Duphaston and control group (100 patients) prescribed GnRH antagonist for the prevention of premature LH surge.
Participants/materials, setting, methods: Both groups received recombinant FSH from third day of menstruation cycle. When follicular diameter reached to14 mm, Cetrotide was prescribed in control group, while Duphaston (20 mg/d) was taken orally from the third day of the cycle until the trigger day in case group.when three dominant follicles reached to17 mm, Oocyte retrieval was performed.Level of hormones in the third day of menstruation, and characteristic of follicles, oocytes, and embryos were compared between two groups
Main results and the role of chance: Results showed that Duphaston successfully inhibits a premature LH surge. There was no significant difference in the level of FSH, estradiol, and LH between the case and control groups ($p>0.05$). Results revealed also showed that Duphaston causes more oocyte retrieval in compared with Cetrotide ($p=0.04$). Although, number of follicles above 14mm, mature oocyte (MII), the total number of viable embryos, i was slightly higher n the case group, but did not reach a significant difference compared with the control group ($p > 0.05$).
Limitations, reasons for caution: . Lack of pregnancy and neonatal outcomes, reduced power of this study. Therefore a complete additional studies is needed to provide more evidence about the efficacy of the Duphaston protocol and illuminate its impact on pregnancy and children born from this novel regimen.
Wider implications of the findings: Duphaston could be used as an appropriate medication instead of GnRH antagonists in women undergoing controlled ovarian hyperstimulation (COH). Duphaston prescription not only prevents premature LH surge, but also improves number of retrieved follicles.
Trial registration number: not applicable

P-709 A comparative study of oral dydrogesterone with micronised vaginal progesterone for supporting the luteal phase in IVF-ICSI cycles

P.C. Jindal[1], M. Singh[1], R. Singh[1]

[1]Bhopal Test Tube Baby Centre, Infertility, Bhopal, India

Study question: Is oral dydrogesterone administration as effective as micronised vaginal progesterone for luteal-phase support in IVF-ICSI cycles ?
Summary answer: Yes , oral dydrogestrone is as effective as micronised vaginal progesterone for luteal-phase support in woman undergoing IVF-ICSI cycles .
What is known already: In ART cycles , there is a significant reduction in pregnancy rates without luteal-phase support . In the absence of luteal-phase support, progesterone levels are suboptimal and accompanied by premature luteolysis, short luteal phase and early bleeding. Progesterone is necessary for implantation.and early pregnancy maintenance.

Luteal phase support is mandatory in assisted reproductive technologies (ART) for optimizing outcome, so the luteal phase is supported with either progesterone, addition of estradiol to progesterone, hCG or gonadotropin releasing hormone (GnRH) agonists. Supplementation of luteal phase with progesterone is prescribed for women undergoing routine IVF-ICSI treatment.
Study design, size, duration: This is a prospective trial in Bhopal from Dec 2018 to Dec 2019. The study protocol was explained for all patients and informed written consents were given. In total 60 infertile women undergoing controlled ovarian stimulation for IVF-ICSI treatment (fresh cycle) were included in this study. Patients were divided into group A (oral dydrogesterone group) and group B (micronised vaginal progesterone group) and outcome evaluated in terms of clinical pregnancy and miscarriage rates.
Participants/materials, setting, methods: In total 60 infertile women undergoing controlled ovarian stimulation for IVF-ICSI treatment (fresh cycle) were included in this study.

Patients were divided into group A (oral dydrogesterone group) and group B (micronised vaginal progesterone group) and outcome evaluated in terms of clinical pregnancy and miscarriage rates.

Group A (n=30) received 10 mg dydrogesterone thrice a day (Duphaston; Abbott) and group B (n=30) received 400 mg micronised vaginal progesterone twice per day.
Main results and the role of chance: Clinical pregnancy rate in the micronised vaginal progesterone (group B) was higher than oral dydrogesterone (group -A) , but the difference was not significant.

Furthermore , the miscarriage rate in two groups was the same .

The difference between the two groups in the endometrial thickness and number of embryos transferred was not significant .

The clinical pregnancy rate and implantation rate were also similar in the two groups. Moreover, no discomforts following oral delivery of Duphaston have been observed during our investigation.

In summary, the use of oral dydrogesterone avoids the frequently reported and negatively perceived side effects of vaginal preparations, whereas no systemic tolerability difference from micronized vaginal progesterone has been identified.

Given the widespread preference of women for an oral compound, dydrogesterone may well become the new standard for LPS in fresh embryo transfer IVF cycles.Limitations, reasons for caution: Very few studies have compared the advantages of oral dydrogestrone with vaginal progesterone for luteal support in ART cycles.

The main limitation of our study was the relatively small sample size. Further investigations are recommended with longer follow-up and larger series to validate the findings reported here.
Wider implications of the findings: Recently, large studies have firmly establish the noninferiority in efficacy of daily 30 mg oral dydrogesterone versus daily 600 mg micronized vaginal progesterone. Despite oral administration and first pass through the liver, dydrogesterone was as well tolerated as vaginal progesterone in safety analyses with no new fetal safety concerns.
Trial registration number: not applicable

P-710 Predictors of pregnancy in women age 40 and above undergoing intrauterine insemination

G. San Roman[1], B. Abittan[1], B. Zaghi[2], R. Goldman[1], S. Brownridge[1], G. San Roman[1]

[1]Northwell Health Fertility, Obstetrics and Gynecology, Manhasset- NY, U.S.A. ;
[2]Southside Hospital- Northwell Health, Obstetrics and Gynecology, Bay Shore- NY, U.S.A.

Study question: Do follicle-stimulating hormone (FSH) and/or anti-mullerian hormone (AMH) levels predict pregnancy rates in women age 40 and older undergoing intrauterine insemination (IUI)?
Summary answer: FSH is a better prognostic indicator for the likelihood of achieving a clinical pregnancy than AMH in women age 40 and above undergoing IUI.
What is known already: IUI is widely accepted as a treatment for infertile couples. Studies have shown decreased pregnancy rates with IUI in women over the age of 40. Thus, the American Society for Reproductive Medicine (ASRM) has recommended in vitro fertilization (IVF) for this age group. However, many patients prefer IUI treatments as these cycles are less-invasive and more affordable than IVF.

Study design, size, duration: We designed a retrospective cohort study of 899 IUI cycles from 2013 to 2019.
Participants/materials, setting, methods: This study was conducted in a tertiary academic medical center. Data was collected from women age 40 and above. Women with tubal or severe male factor infertility were excluded from this study. Two-sample two-tailed t-tests assuming unequal variance were used to assess the differences in FSH and AMH levels between the pregnant and non-pregnant groups.
Main results and the role of chance: Of the 899 IUI cycles in our study, 51 (5.8%) resulted in pregnancy. No pregnancies were reported in women over the age of 45 (n=67). FSH levels among patients who achieved clinical pregnancy were significantly lower (8.7 IU/L ± 4.1) than those of patients who did not conceive (10.7 IU/L ± 6.6; *p*=0.002). AMH levels were not significantly different between the pregnant and non-pregnant patients (1.5 ng/mL ±1.7 vs. 1.5 ng/mL ± 3.2; p=0.87).
Limitations, reasons for caution: One of the limitations of our study is that this was a retrospective chart review. Given the retrospective nature of this study, specific patient indication and desires for undergoing IUI treatments as opposed to IVF were not readily available.
Wider implications of the findings: Patients age 40 and over who request infertility treatment should have detailed counseling of the likelihood of success relative to their age. Our study shows that IUI may be an option for women who do not want or cannot afford IVF.
Trial registration number: not applicable

P-711 Double stimulation protocol (DuoStim) might improve pregnancy rate in poor responders

M. Ibrahim[1]

[1]*menia university faculty of medicine, obstetrics and gynecology departement, menia, Egypt*

Study question: Is double stimulation protocol superior to short protocol in improving the pregnancy rate in poor responders?
Summary answer: Stimulation with identical protocol in the Follicular phase and Luteal Phase of the same menstrual cycle resulted in more number of blastocysts in poor responseders.
What is known already: The number of high quality oocytes retrieved in an IVF cycle is a major factor contributing to the patient pregnancy chances . While stimulation and trigger concepts have been developed successfully in normo and hyperrespoder patients the poor responder patients remains difficult to manage. However, recent advances in definitions and classifications of poor ovarian responders might enable a more accurate and clinically useful interpretation of new treatment concepts.
Study design, size, duration: Prospective cohort study done on fifty patients from December 2018 to December 2019
Participants/materials, setting, methods: Nile infertility center(NIC) and Minia Infertility Research Unit (MIRU).
Fifty poor responders planned for ICSI.
Method
1st group using the standard Long protocol for controlled ovarian hyperstimulation
2nd group using Double stimulation protocol (DuoStim) we starting from the 2nd day of the cycle with 300 IU of r- FSH + 150IU of r-LH using flexible antagonist protocol and triggering by GnRH-a then OPU after 36 hours.
Main results and the role of chance: Patients with an antimüllerian hormone level of <0.5 ng/mL, antral follicle count of <5 follicles, and/or <3 oocytes retrieved in a previous cycle were included. There are a statistically significant differences were found in the number of retrieved COCs (2.6 ± 1.8vs. 5.2 ± 3.4), MII oocytes (1.4 ± 1.2 vs. 3.9 ± 2.3) and number of transferred blastocysts(1.1 ± 0.9vs. 2.2 ± 0.8)also there is statistically significant difference in clinical pregnancy rate between the two groups (12% vs 20%) there is no statistically significant difference were found in the multiple pregnancy rate(1% vs1%) .
Limitations, reasons for caution: high cost of the treatment used limit the study
Wider implications of the findings: We recommend to implicate the study on larger sample size.
Trial registration number: not applicable

P-712 Granulosa cell signaling-based bioassay in vitro for personalized stimulation in ART: benefit of LH addition to FSH in sub-responder women

E. Paradiso[1], **C. Lazzaretti**[1], **S. Sperduti**[1], **S. Limoncella**[1], **B. Melli**[1], **M.T. Villani**[2], **A. Nicoli**[2], **J. Daolio**[2], **M. Lispi**[3], **G. Orlando**[4], **M. Simoni**[1], **L. Casarini**[1]

[1]*University of Modena and Reggio Emilia, Unit of Endocrinology- Dept. Biomedical- Metabolic and Neural Sciences, Modena, Italy ;*
[2]*Azienda Unità Sanitaria Locale – IRCCS di Reggio Emilia- Reggio Emilia- Modena, Department of Obstetrics and Gynaecology- Fertility Center- ASMN., Reggio Emilia, Italy ;*
[3]*Merck KGaA, Global Medical Affair, Darmstadt, Germany ;*
[4]*Merck Serono SpA, Medical Affair, Roma, Italy*

Study question: Can LH addition to FSH recover the difference of granulosa cell cAMP and progesterone response between sub- vs normo-responder women?
Summary answer: Co-treatment by recombinant FSH+LH may reduce, or even deplete the different cAMP and progesterone production between granulosa cells from normo- and sub-responder women.
What is known already: Granulosa cells collected from 105 normo- and 105 sub-responders women undergoing assisted reproduction (ART) feature specific FSH receptor (FSHR) expression and response to gonadotropins *in vitro*. Indeed, we demonstrated previously that normo-responders display higher sensitiveness to FSH than sub-responders, resulting in lower FSH 50% effective concentrations (EC50) required for inducing cAMP and steroid response. Moreover, normo-responders have higher basal serum estradiol levels than sub-responders.
Study design, size, duration: hGLC samples from anonymized donor women undergoing oocyte retrieval for ART are collected between 2019-2020. Cells are purified, cultured, genotyped and treated by FSH+LH *in vitro* and intracellular signaling endpoints were collected. Data obtained by cells from 42 normo-responder women are compared to those from 24 sub-responders (women who recruited a number of oocytes <70% than Follicular Output Rate). Experiments are performed under the local Ethics Committee permission (n°2018/0080377, 16/07/2018) and written consent.
Participants/materials, setting, methods: hGLC donor women undergone ART without endocrine abnormalities and diseases. hGLC samples are genotyped for FSHR polymorphisms known to modulate the response to FSH (rs6166, rs6165, rs1394205) and stimulated by increasing doses (0-100 nM) of recombinant FSH (Gonal-F, Merck KGaA, Darmstadt, Germany) + 10 nM LH (Luveris, Merck KGaA). cAMP and, as a primary endpoint, progesterone are measured by immunoassays and dose-response curves performed. Results from normo vs sub-responders were compared, considering their clinical data.
Main results and the role of chance: We found that cells of sub-responder women treated with FSH+LH produced higher cAMP levels than same cells treated by FSH alone. The presence of LH compensate for the different FSH dosage, required for increasing cAMP, between normo- and sub-responder cells (FSH EC50 2.668 ± 1.012 vs 1.486 ± 1.075 nM, respectively; p<0.01; t-test). Then progesterone synthesis was evaluated, indicating activation of the steroidogenic pathway. The 24-h basal progesterone production of cells from normo-responder women is higher than that of cells from sub-responders (p<0.01; t-test). In the presence of 10 nM LH, both unstimulated and FSH-induced, progesterone production increased in all samples. Interestingly, although progesterone produced by cells from normo-responder women is confirmed to be higher than that of cells from sub-responders (about 2-fold different progesterone levels; p<0.01; t-test). FSH (+ LH) EC50 values are similar in the two groups, revealing that the addition of LH is linked to equipotency of FSH in inducing steroidogenesis, in the two groups (t-test; p≥0.05). No different allele frequencies and FSHR expression levels between normo- versus sub-responders were found (Chi square and t-test; p>0.05), demonstrating that each of the two groups are genetically homogeneous.
Limitations, reasons for caution: In vitro data would be confirmed by translation into a clinical setting.
Wider implications of the findings: This study provides the rationale for establish an *in vitro* bioassay able of inferring and optimizing the *in vivo* response to ART treatment, evaluating steroidogenesis. The model suggests that LH addition to FSH treatment for ovarian stimulation may be beneficial in sub-responder women.
Trial registration number: not applicable

P-713 Severe haematoperitoneum after transvaginal oocyte retrieval related ovarian bleeding could be mostly managed by conservative treatment: 9577 cases of one clinician's experience in 5.5 years

M. Aygun[1], C. Pirkevi Çetinkaya[1], M. Cetinkaya[1], B. Yuksel[1], S. Kahraman[1]

[1]Sisli Memorial Hospital, IVF Andrology and Genetics Center, Istanbul, Turkey

Study question: How can we manage the severe haematoperitoneum (SHP) caused by ovarian bleeding after transvaginal oocyte retrieval (TVOR), surgical or conservative?
Summary answer: Most of the patients does not need surgery for TVOR-related intra-abdominal severe bleeding after red blood cell (RBC) transfusion and clinical observation.
What is known already: One of the most serious complications of the TVOR is intra-abdominal bleeding during in-vitro fertilization (IVF) cycles. Hospitalization is reported to be needed after this surgical procedure with an incidence between 0.06% and 0.35%. According to the literature, the rate of cases with suspected severe SHP requiring abdominal surgery is very different between 40% to 70% in large series. However, very little data on surgical versus conservative treatment for SHP caused by ovarian bleeding after transvaginal egg retrieval is currently available.
Study design, size, duration: A total of 9577 consecutive TVOR procedures performed by a single clinician were included in this retrospective study over a period of 5.5 years between June 2014 and December 2019 in one IVF center. All of the suspected SHP cases who were hospitalized were enrolled in the study group. This "complication" group was categorized according to the need for conservative or surgical treatment. General SHP rates and the treatment approaches were compared.
Participants/materials, setting, methods: The patients of suspected SHP were hospitalized and evaluated. Patients with non-ovarian bleedings were excluded. The study was grouped into two. Group I included patients in whom conservative treatment with or without RBC transfusion was performed; Group II consisted of patients who were indicated for surgical treatment. After discharge from hospital, the patients were followed up for approximately 12 weeks.
Main results and the role of chance: 9577 IVF patients who came for TVOG were performed by the same clinician between June 2014 and December 2019. A total number of 90190 oocytes were retrieved and the mean number of retrieved oocytes was 9.41±8.26. The number of SHP related ovarian bleeding complications during TVOG was 20 out of 9577 (0.20%). Whereas 17 patients (85%) needed only conservative treatment, only 3 patients (15%) needed a laparoscopic intervention. Hemostasis control method for ovarian bleeding in laparoscopy was electrocoagulation in one patient and suture in another two. None of the patients (20) had severe infections such as pelvic abscess or sepsis after the treatment. One patient was excluded from the study due to retroperitoneal bleeding.
Limitations, reasons for caution: Although all complications were treated in our institution by one surgeon, we do not know the real impact of complications and their related treatments on further infertility treatments. This could reflect a possible bias on the best solution regarding treatment methods of severe ovarian bleeding complication related to TVOR.
Wider implications of the findings: Although TVOR is a very standard procedure in IVF, the real complication rates and their treatment methods are seldom published. New international registries and databases are needed for outcomes and managements of the complications of TVOR.
Trial registration number: none

P-714 Lipid peroxidation and coenzyme Q 10 levels in follicular fluid of women undergoing IVF: a comparison between poor and normal ovarian responders

P. Sirayapiwat[1], P. Thuwanut[1], N. Orprayoon[1], R. Rungsiwiwut[2], W. Sereepapong[1]

[1]Chulalongkorn University, Obstetrics and Gynecology, Bangkok, Thailand ;
[2]Srinakharinwirot University, Department of Anatomy, Bangkok, Thailand

Study question: Is oxidative stress (lipid oxidation) in follicular fluid one of crucial factors of poor ovarian response (POR)?
Summary answer: The level of lipid peroxidation in follicular fluid showed no significant difference, whereas the antioxidant coenzyme Q10 level was significantly lower in POR group.
What is known already: Poor response to controlled ovarian hyperstimulation is one of major obstacles of successful in vitro fertilization (IVF) outcomes. In older women, age-dependent decline in ovarian reserve and oocyte quality attributes to poor ovarian response, while an underlying cause in younger women is still enigmatic. One of the possible mechanisms are oxidative stress and mitochondrial dysfunction. Coenzyme Q10 (CoQ10) is a lipid-soluble coenzyme which is an essential component of the inner mitochondrial membrane. It plays a pivotal role in intracellular antioxidant by inhibiting lipid peroxidation and DNA oxidation.
Study design, size, duration: A cross-sectional analytic study, follicular fluid from 28 women with normal ovarian response (NOR) and 28 patients with POR to controlled hyperstimulation (COH) were collected between September 2018 and January 2019 in a single academic center.
Participants/materials, setting, methods: Fifty-six infertile women, age between 25-45 years-old, without active ovarian pathology, basal FSH < 12 IU/L, AMH > 1.0 ng/ml who underwent IVF, were recruited into this study with 28 participants in POR group (1-4 retrieved oocytes) and 28 participants in NOR group (5-15 retrieved oocytes). The follicular fluid from the first punctured follicle were compared for the levels of CoQ10 and the end product of lipid peroxidation (LPO), malondialdehyde, by ELISA.
Main results and the role of chance: The mean age of participants was 39.1 year (range 32-43) in POR group and 37.1 year (range 29-42) in NOR group. The mean number of retrieved oocytes was 3.0+0.9 in POR group and 7.7+2.6 in NOR group. The levels of follicular fluid CoQ10 of women in POR group were significantly lower (0.48 + 0.31 nmol/mL) than in NOR group (1.23 + 0.85 ng/mL), $p < 0.001$, whereas the LPO levels was not significantly different between two groups, 10.34+5.88 nmol/mL in POR group and 8.25+2.82 nmol/mL in NOR group, respectively ($p = 0.09$). The clinical pregnancy rate was 17.86% and 35.71% in POR and NOR group, respectively.
Limitations, reasons for caution: The end products of lipid peroxiadation other than malondialdehyde were not analysed in this study. Other limitation of this study includes wide range of participants' ages.
Wider implications of the findings: Our results demonstrate low level of antioxidant CoQ10 in follicular fluid as a potential underlying mechanism of poor ovarian response. However, further investigation for other pathways of oxidative stress in follicular fluid and cumulus-oocyte complex are necessary to elucidate.
Trial registration number: ClinicalTrials.gov Identifier: NCT03155438

P-715 The role of vitaminD in human follicular fluid - Exploratory biochemical analysis of biomarkers: correlation of 25-hydroxyvitaminD and active 1,25-dihydroxyvitaminD between serum and follicular fluid

S. Leser[1], B. Greene[2], L. Witzke[1], M. Nesbigall[1], P.I. Pfefferle[3], U. Wagner[1], V. Ziller[1]

[1]University hospital Gießen and Marburg UKGM, Department for gynecology and obstetrics- Division of gynaecological endocrinology and reproductive medicine, Marburg, Germany ;
[2]Philipps University Marburg, Medical Biostatistics and Bioinformatics, Marburg, Germany ;
[3]University hospital Gießen and Marburg UKGM, Comprehensive Biobank Marburg, Marburg, Germany

Study question: What is the role of active Vitamin D-hormone (1,25-dihydroxyvitamin D) in human follicular fluid? Do concentrations of vitaminD differ from serum as a sign of distinct active follicular vitaminD synthesis?
Summary answer: Follicular fluid (FF) concentrations and serum concentration of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D showed a highly significant correlation.
What is known already: Obtained as a by-product within the process of oocyte collection FF is predestined as a subject of research in assisted reproduction. In order to establish contained components as oocyte quality markers its examination is of high value. 25-hydroxyvitamin D is one of them, playing a key role in human reproduction. However, the results are conflicting and mostly pooled FF is measured so far. The scientific findings about the role of

1,25-dihydroxyvitaminD as the biologically active form of vitaminD are poor. None of the previously published studies adopted the strategy of separate collection and analysis of the FF of single follicles.
Study design, size, duration: In a prospective exploratory study, 20 infertile women undergoing IVF between January 2018 until December 2019 were included. Written permission was required. Known illnesses of the reproductive system and infectious diseases were defined as exclusion criteria. In the end a total case number of 58 follicles were analyzed.
Participants/materials, setting, methods: During the oocyte collection the sizes of follicles were measured and FF of a variable number of single follicles, according to the total number of follicles per patient and their size, was separately collected. Serum samples were obtained on the same day. After centrifugation all samples were cryopreserved at -80°C and analyzed for their 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D content after thawing.
Main results and the role of chance: The levels of 25-hydroxyvitamin D in serum and FF were significantly and strongly correlated (r=0.82, p=0.001) but hardly differed (31.00 (29.15-32.86) versus 29.62 (27.90-31.33)ng/ml, p=0.001). In addition we determined that the mean concentration of 1,25-hydroxyvitamin D is slightly but significantly higher in serum than in FF (53.53 (50.13-56.94) versus 45.51 (42.42-48.61)ng/ml, p=0.001, r=0.44). Regarding the concentration of vitamin D, significant modification of the composition of the FF as an ultra-filtrate of the serum by the follicular granulosa and theca cells does not seem to occur. There was no evidence for 1,25-dihydroxy vitamin D synthesis within the follicles.
Limitations, reasons for caution: The process of follicle separation is not 100% exact, however as other markers studied showed marked differences the gained data appears reliable. IVF stimulated cycles may not represent the situation in natural cycles, further investigations are needed.
Wider implications of the findings: As vitamin D and reproduction, as well as supplementation in fertility patients is an important part of current medical and scientific discussions the correlation of our findings with clinical outcome parameters are planned to gain further insights in the mechanisms of vitamin D and its role in the assisted reproduction.
Trial registration number: n/a

P-716 Repeat ovarian stimulation cycles in oocyte donors: Results from a cohort study

A. Zavala-Garcia[1], B. Lozano-Moreno[2], M.G. Juan Manuel[3], R. Lopez-Sanchez[4], J.J. Lopez-Galvez[1]

[1]UR Clínica Vistahermosa, Unidad de Reproducción, Alicante, Spain ; [2]UR Clínica Vistahermosa, Assisted Reproduction Medicine and Genetics Masters Program- Miguel Hernandez de Elche University, Alicante, Spain ; [3]Unidad de Reproducción UR Clínica Vistahermosa, Biology of Human Reproduction and Clinical Embryology Laboratory, Alicante, Spain ; [4]UR Clínica Vistahermosa, Biology of Human Reproduction and Clinical Embryology Laboratory, Alicante, Spain

Study question: Are there changes in total FSH dose, follicular count, retrieved meiosis metaphase II (M2) oocytes and pregnancy rates in up to 4 repeated donor cycles?
Summary answer: There are no major statistically significant changes in FSH dose, follicular count, retrieved meiosis metaphase II (M2) oocytes and pregnancy rates in repeated donor cycles.
What is known already: The number of patients requiring oocyte donation is thought to be greater than the number of eligible donors; which is a trend that will most likely keep growing within the following years. Recruiting the same donor multiple times is a way to overcome this demand, which is why the need to further investigate effects of repeated stimulation cycles of oocyte donation. Optimizing donor recruitment and understanding how their characteristics influence outcomes in recipients is essential. Even though a handful of studies have explored factors associated to pregnancy from OD cycles with contradictory results, they've studied few cycles in small populations.
Study design, size, duration: We performed a retrospective cohort study from January 2012 to October 2019. The study population consisted of women who underwent at least 4 stimulation protocols for oocyte donation, for a total of 47 women and 188 ovarian stimulation cycles. We performed mean, standard deviation, and for inferential analysis: Shapiro test, and Student's T. For categorical variables Chi Squared.
Participants/materials, setting, methods: The sample size for this study was based on the number of patients recruited at the Unit of Reproduction *UR Clínica Vistahermosa*, in Alicante, Spain; a private, tertiary reference center. Inclision criteria: (1) women 18-35 years, (2) no primary or secondary infertility, (3) seeking to become an egg donor, (4) not pregnant, (5) conventional stimulation protocol, (6) at least four stimulation cycles.
Main results and the role of chance: A total of 211 patients (525 cycles) composed our cohort with 47 women who underwent at least four consecutive stimulation cycles for egg donation, amounting to a total of 188 stimulation cycles. A total of 96 donors underwent at least one stimulation cycle, while the maximum number of repeated stimulation cycles was 12, performed on one donor. The mean age was of 24.09±3.70 years, 93.61% were of normal weight, with only 6.38% overweight and none within an obesity range BMI. Most had proven fertility or past pregnancy (71.27%) and most were active tobacco consumers, 64.89%. Donor age varied within cycles,as 22.89±3.59 in the first cycle, 23.81±3.66 in the second, 24.28±3.57 in the third, and 25.36 ±3.64 in the fourth. We compared each result aginst the baseline (first cycle) result, and only the average age in the last cycle was statistally significt (P=0.001). Total FSH dose was only statistically significant in the third vs baseline (2168.62±795.08 VS 1890±561.61, respectively, P=0.003). Total oocytes and M2 oocytes did not vary between cycles. Pregnancy rates were not statistically significant between cycles and their percentage was 59.75, 51.06, 51.06 and 53.19% for the first, second, third and fourth cycle, respectively.
Limitations, reasons for caution: The limitation of our study is a small population.
Wider implications of the findings: Optimizing donor recruitment and understanding how their characteristics influence outcomes in recipients is essential as the demographics in populations continue to trend towards a greater ageing population and fertility continues to decline, which is why the importance of understanding donor outcome is essential.
Trial registration number: NA

P-717 follicular tracking with ultrasound does not improve pregnancy rates in letrozole intrauterine insemination (IUI) cycles

K. Cho[1], J. Au[2], R. Sen-Laurenz[3], A. Albert[4], J. Havelock[1], C. Dunne[1]

[1]University of British Columbia, Department of Obstetrics and Gynecology-Division of Reproductive Endocrinology & Infertility, Vancouver, Canada ; [2]Pacific centre for Reproductive Medicine, Department of Embryology, Burnaby, Canada ; [3]University of British Columbia, Department of Sciences, vancouver, Canada ; [4]Women's Health Research Institute, Department of Biostatistics, Vancouver, Canada

Study question: In letrozole intrauterine insemination (IUI) cycles, is the probability of pregnancy associated with ultrasound monitoring or lead follicle size?
Summary answer: Ultrasound monitoring was not associated with an increased pregnancy rate; the optimal follicle size was 18-19.99mm in the unadjusted analysis.
What is known already: The worldwide shortage of clomiphene has shifted practice towards the use of letrozole for ovulation induction and unexplained infertility. However, the current protocols for letrozole IUI cycles are still largely based on the studies using clomiphene. Previous research has shown that ultrasound monitoring did not improve pregnancy outcomes with clomiphene IUI. Furthermore, a lead follicle size ≥20-23 mm has been suggested as the optimal cut-off for ovulation triggering with clomiphene. This criterion has been extended to those using letrozole.
Study design, size, duration: This retrospective cohort study includes letrozole cycles with IUI performed at a university-affiliated fertility clinic from 2016 to 2019. Exclusion criteria included: anovulatory cycles, tubal factor infertility, or total motile sperm < 1million. The patients were divided into 2 groups: ultrasound monitored and unmonitored. The associations between pregnancy rate and ultrasound use, lead follicle size at last ultrasound, endometrial thickness, or human chorionic gonadotrophin (HCG) use were determined using logistic regression and Fisher's exact test.

Participants/materials, setting, methods: Letrozole 2.5-7.5 mg was administered from cycle day 3-7 or day 1-9. In the unmonitored group, patients used ovulation predictor kits(OPK) daily starting on cycle day 10. In the ultrasound monitored group, patients presented for an ultrasound at baseline, on day 10-12, and as needed until ovulation. HCG 10,000IU was given for ovulation trigger when clinically indicated. The IUI was performed on the same day as a positive OPK or the day after HCG trigger.

Main results and the role of chance: Out of the 1196 cycles undertaken by 620 patients, 136 cycles (11.4%) were monitored with ultrasound. Overall, the pregnancy rate was 14.4% per cycle. Ultrasound monitoring was not associated with increased pregnancy rates in letrozole IUI treatments (p= 0.52).

Adjusting for age and body mass index, there was no linear relationship between lead follicle size and the odds of pregnancy (OR 1.04, 95% CI 0.86 to 1.27, p= 0.68). Multi-follicular response was also not significantly associated with an increased pregnancy rate; the presence of 2 or more lead follicles ≥18mm had 3.12 adjusted odds of pregnancy (0.85 to 10.61, p= 0.08). There was no relationship between endometrial thickness and pregnancy (OR = 1.01, 95% CI 0.79 to 1.26, p = 0.96). Similarly, the addition of HCG to trigger ovulation was not associated with increased rates of pregnancy (p= 0.58).

There was a significantly decreased pregnancy rate in those with a lead follicle measuring ≥20mm in mean diameter compared to those with lead follicle size 18-19.99mm (9.6% vs 33.7%, p= 0.005). However, there are too few pregnancies to analyze follicle size as a categorical variable for logistic regression.

Limitations, reasons for caution: While there is a statistically significant decrease in the pregnancy rate associated with lead follicles ≥20 mm, caution must be taken prior to altering clinical care. Due to the small sample size (n=22) of pregnancies in this group, these findings can also occur as a result of random sampling.

Wider implications of the findings: Overall, there is no significant relationship between ultrasound monitoring and pregnancy in letrozole IUI. However, in the subset of patients who require monitoring for accurate IUI timing, there is evidence to suggest that the optimal follicular size may be 18-19.99mm. A larger sample size is needed to confirm these findings.

Trial registration number: not applicable

P-718 Variation in luteinizing hormone levels in artificially prepared frozen-thawed embryo transfer cycles: impact on live birth rates

J. Siffain[1], A. Racca[1], P. Drakopoulos[1], B. Popovic-Todorovic[1], C. Roelens[1], S. Mackens[1], H. Tournaye[1], M. De Vos[1], H.M. Fatemi[2], C. Blockeel[1]

[1]UZ Brussel, Reproductive Medicine, Jette- Brussels, Belgium ;
[2]IVIRMA Middle East Fertility Clinic, Fertility, Abu Dhabi, United Arab Emirates

Study question: Does the variation in luteinizing hormone (LH) affect endometrial receptivity and consequently live birth rates (LBRs) in artificially prepared frozen embryo transfer cycles (FET)?

Summary answer: LBRs following artificially prepared FET cycles are not affected by variations in LH levels between the start and the end of the proliferative phase.

What is known already: Luteinizing hormone binds the LH/choriogonadotropin receptor (LHCGR) in the ovaries to promote luteinization and progesterone production. Previous studies have demonstrated the presence of LHCGR in extra-gonadal tissues, including the endometrium, where LH is thought to influence cell metabolism and growth by increasing the local production of progesterone (P). Additionally, LH may play a role in embryo implantation. As a result a positive feedback mechanism from exogenous administrated estrogens in HRT-FET cycles, increased circulating LH levels may potentially affect pregnancy outcomes.

Study design, size, duration: This is a retrospective study performed at a tertiary university-based hospital. A total of 1445 autologous HRT-FET cycles between 2010 and 2016 were included in the study. To avoid bias, we only selected patients who performed an in-house blood sample to assess LH levels across the HRT-FET cycle.

Participants/materials, setting, methods: LBRs were compared among three interquartile ranges for the difference in LH value (delta LH) at start of E2 supplementation and at the end of the proliferative phase. More specifically, we compared p<25 (delta LH<2.5 IU/L), p25-75 (delta LH 2.5- 11.3 IU/L), >p75 (delta LH >11.3 IU/L). A regression model with estimation by generalized estimating equations (GEE) was used to adjust for patients performing more than one FET cycle, as well as for known confounders.

Main results and the role of chance: Baseline demographic and clinical characteristics were comparable among the three groups except for BMI and PCOS. BMI was significantly lower in the p<75 group (25.2 kg/m2, 25.0 kg/m2 and 23.5 kg/m2 respectively for the p<25, p25-p75 and p>75 group, p<0.001), while PCOS was more common in the p<25 group (respectively, 28.5%, 22.8% and 20.6%, p=0.034), as well as the presence of irregular cycles (respectively, 59.3%, 37.8% and 32.2%, p<0.001). LBRs per started cycle were not significantly different between the investigated groups and were respectively 19.9%, 22.7% and 20.1% for the p<25, p25-75 and p>75 group (p=0.524). Miscarriage rates, on the other hand, were similar among the groups (10.83%, 10.10% and 9.76% for the p<25, p25-75 and p>75 group, respectively p=0.88). According to GEE, accounting for the following confounding factors: age, BMI, presence of irregular cycles, duration of E2 supplementation, level of serum progesterone at the end of the proliferative phase, delta LH was not significantly associated with LBR (coefficient 0.005, 95% CI- 0.02 to 0.03, p=0.72). These results were replicated in case of substantially large delta LH (p>95), as well as, when late follicular LH levels were considered as a continuous variable and failed to show any association with LBR.

Limitations, reasons for caution: Although this study included a large number of cycles and a GEE was performed to adjust for multiple cycles per patient, the results remain limited by the retrospective nature of the study and its associated bias.

Wider implications of the findings: The variation in LH between the start and the end of the proliferative phase in HRT-FET cycles has no influence on live birth rates. The estradiol-priming induced LH rise is insufficient to have any effect on the treatment outcome. Monitoring of LH levels during HRT-FET cycles is of questionable value.

Trial registration number: not applicable

P-719 Is the intravenous lipid emulsion use related to higher pregnancy and live birth rates in patients submitted to in vitro fertilization?

M. HENTSCHKE[1], L. Marquardt Burmann[1], V. Campos Dornelles[1], T. Colombo[1], V. Devens Trindade[1], A. Kira[1], R. Petracco[1], A. Petracco[1], M. Badalotti[1]

[1]Pontifícia Universidade Católica do Rio Grande do Sul PUCRS, School of Medicine, Porto Alegre, Brazil

Study question: Is the intravenous lipid emulsion (ILE) use associated with higher pregnancy rates in patients with implantation failure history after *in vitro* fertilization?

Summary answer: Patients who received more than one dose of ILE presented higher pregnancy and live birth rates than those who receive only one dose.

What is known already: Recent studies have shown benefits of using ILE in patients with repeated implantation failure, due to its ability to inhibit Natural Killers cells activity - whose exacerbated activity could be harmful in the implantation process.

Study design, size, duration: Retrospective cohort study performed at an assisted reproduction clinic in southern Brazil, that included 359 patients, with at least 2 embryo transfer failures. Data were collected from electronic record from April/2017 to February/2019.

Participants/materials, setting, methods: Samples were divided into two groups: Group 1, patients who received ILE infusion (n=64) and Group 2, who didn't receive ILE (n=295). Group 1 received 2ml of 20% ILE infusion. After pregnancy confirmation with positive hCG, some patients received a second and/or third dose of the medication. Data were presented as mean ± standard deviation or frequency and percentage. For statistical analysis, Student's test or Fisher's exact test and Pearson correlation were used, considering p<0.05.

Main results and the role of chance: Comparing ILE group *vs.* Control group, the following results were found: maternal age (36.9±4 *vs.* 36.6±3.7, p=0.711), paternal age (40.3±5.3 *vs.* 39.9±6.3, p=0.474), biochemical pregnancy (50% *vs.* 41.5%, p=0.141), clinical pregnancy (45.3% *vs.* 37.6%, p=0.158).

Regarding clinical outcomes, the rates of abortion/ectopic pregnancy were 9.3% *vs*. 2.0%, p=0.010 and live birth rate was 35.9% *vs*. 35.6%, p=0.53. When gestational age, Apgar index, birth weight and length were analyzed, no statistical differences were found. From the 45.3% clinical pregnancies in Group 1, 69% received two or more doses of ILE, leading to a positive correlation between ILE doses and clinical pregnancies variables (r=0.669, p<0.001). When comparing one dose with two or more doses, higher clinical pregnancy and birth rates were observed when more than one dose was used (p<0.001).

Limitations, reasons for caution: Retrospective study based on electronic records. Medications that could have influenced on pregnancy rates, such as aspirin and heparin were not analyzed in this study.

Wider implications of the findings: This study demonstrates that ILE could be an option for couples seeking IVF treatment with two or more implantation failures. More than one dose of the medication should be encouraged after a positive hCG in order to achieve higher clinical pregnancy and live birth rates.

Trial registration number: NOT APPLICABLE

P-720 A comparison of women's perceptions and acceptability of micronised progesterone/medroxyprogesterone acetate in combination with transdermal oestradiol in the management of young postmenopausal women <45-years

M. Mittal[1], P. Supramaniam[2], H. Hamoda[3]

[1]Imperial College Healthcare NHS Trust, Gynaecology- Reproductive Medicine, London, United Kingdom ;
[2]Oxford University Hospitals NHS Trust, Women's and Childrens, High Wycombe, United Kingdom ;
[3]King's College Hospital NHS Trust, Women's and Childrens, London, United Kingdom

Study question: To assess the acceptability and perception of young menopausal women to HRT containing micronised progesterone (MP) or medroxyprogesterone acetate (MPA), on symptom control and development of adverse effects.

Summary answer: The acceptability of both regimens was high despite adverse effects, but tolerability of MP appeared better with fewer reporting psychological concerns compared to MPA-containing regimens.

What is known already: The primary indication for HRT is symptom relief, with approximately 75% experiencing vasomotor-symptoms. Young postmenopausal women (secondary to premature ovarian insufficiency/early menopause), represent a subset population, whereby the mainstay of management is to provide physiological levels of the deficient hormones and thus, counteract the effects of the long-term sequelae of early menopause. HRT is recommended until at least the average age of the menopause. The majority of data used to counsel these women on the risk profile is extrapolated from studies conducted in older women. Furthermore, progestin is said to be responsible for some of the adverse-effects, limiting compliance.

Study design, size, duration: Prospectively recruited young menopausal women, <45 years with an intact uterus, were randomised to one of two treatment arms for 12-months duration, MP or MPA in combination with transdermal oestradiol delivered in the form of patches. A self-reported questionnaire with matrix rating scales, repeated at intervals of 3, 6 and 12-months from the commencement of the HRT regimen were compared from baseline and between the two treatment arms. A total of 190 questionnaires were returned.

Participants/materials, setting, methods: A matrix scale was used to rank the symptoms/concerns in order of priority. The scores were then grouped to signify their main symptoms (1-3), moderate symptoms (4-7) and mild symptoms (8-10) for analytical purposes.

A matrix scale was also used to rank the severity/impact of the symptom/concern on their quality of life (weighted average). The scores were grouped for analytical purposes: 1-3 signifying minimal impact; 4-7 moderate impact; and, 8-10 maximal impact.

Main results and the role of chance: The most commonly reported symptoms were low energy levels, vasomotor symptoms and sexual dysfunction. The reported symptoms did not always correlate to those that were perceived to have the greatest impact on the individual's quality of life. The prevalence of adverse effects ranged between 57.89%-87.50%. A non-significant reduction in reported adverse-effects was demonstrated in the MP treatment arm (73.91% at 3-months reducing to 57.89% at 12-months duration; p=0.33), compared to the non-significant increase seen in the MPA treatment arm (76.92% at 3-months increasing to 87.50% at 12-months duration; p=0.69). The main reported adverse effects were gastrointestinal symptoms (weight change and bloating) and psychological effects (mood swings and irritability). A significant difference in reported adverse-effects was documented between the groups after set periods, with a significantly greater proportion reporting breast tenderness after 3-months duration (p=0.01), lower numbers reporting mood swings at 6-months duration (p=0.01) as well as irritability at 12-months duration (p=0.03) in the MP treatment arm compared to the MPA treatment arm. Despite the reporting of adverse symptoms, 78.86% would continue to use HRT outside of a research setting with no reported difference in satisfaction between the HRT regimens (p=0.24).

Limitations, reasons for caution: The questionnaire was developed using the 12-points described by Burford et al., (2009). Face validity was also assessed through testing on the target population and quantitative data was obtained to reduce the Hawthorn effect.

Wider implications of the findings: Significant controversy surrounds the use of HRT. Current evidence is derived from an extrapolation of data from older menopausal women. Directed studies in the younger population will help demonstrate acceptability and tolerability in those that would benefit the most from its use.

Trial registration number: REC Number: 12/LO/1957; EudraCT Number: 2012-004511-30

P-721 Double stimulation in the same ovarian cycle might improve the number of competente oocytes and euploid embryos

T. Farias Lima[1], M. Pessoa Caldas[1], P. César Silva Matheus[2], L. Albuquerque Barros[2], C. Pessoa Caldas[3], L. Pessoa Caldas[3], C. Monteiro[1], N. Pimentel Fischer Pacheco[1], A. Eunice[1], R. Mota Paixão de Sousa[1], M. Correa Nunes[1], A. Griz[1], T. Matheus[1], L. Helena Amorim[2], M. Almeida[2]

[1]Geare Group IVF, Reproductive specialist, Recife, Brazil ;
[2]Geare Group IVF, Embriologist, Recife, Brazil ;
[3]Maurício Nassau university center, Medical Student, Recife, Brazil

Study question: Is the Double Stimulation a interesting protocol to enlarge the numbers of competent oocytes and euploid blastocysts in the group of poor responder patients?

Summary answer: The Double stimulation aproach maximizes the number of recovered oocytes and euploid embryos, possible increasing the rate of clinical pregnancy and live births.

What is known already: GnRH antagonists, estradiol priming, double stimulation, letrozole administration, DHEA, and herbal therapy supplementations are the recent modifications done to improve oocyte retrieval and subsequent embryo transfer for POR cases. The DuoStim strategy is promising to manage the chalenge population of poor responders, especially to avoid drop-out after a first failed attempt.

Study design, size, duration: We report here our 1-year experience of Duostim practice in our private IVF Center including five clinics, in january to december of 2019, in a total of 709 cases that year. Patients included were selected to the group of poor responders (POR) using the POSEIDON group definitions. The financial barrier is a problem reported by patients to perform DuoStim cycles.

Participants/materials, setting, methods: This is a retrospective analysis of forty poor prognosis patients who completed a DuoStim protocol. Thirty-three patients underwent IVF with blastocyst-stage and seven patients gone to oocyte vitrification for social preservation. The mean age was 38,6 years. The IVF cycles were followed by preimplantation genetic testing for aneuploidies.

Main results and the role of chance: The folicular phase stimulation (FPS) and luteal phase stimulation (LPS) was realized with a maximum dose of 300 IU of recombinant FSH and recombinant LH 150 IU with the same gonadotropin releasing hormone (GnRH) antagonist protocol, agonist trigger with GNRHa (leuprolide 0,2mg), and intracytoplasmic sperm injection with ejaculated sperm. Regarding the number of oocytes collected and oocyte maturation, in 20/40 cases the LPS was better, the FSP was superior in 15/40 and in 5/40 cases it

was similar in both phases. About the rate of blastocysts, it was similar in the two phase stimulations in 13/33 cases, the result of FPS was better in 10/33 cases and the LPS was in 10/33. The rate of euploidy in LPS in 4/33 cases was better, 6/33 cases were similar and in 12/33 cases the result in FPS was better. In this regard, LPS seems to contribute to conventional stimulation with more oocytes with a comparable competence as FPS, retrieved per ovarian cycle. The mean numbers of euploid blastocysts obtained from the cohorts of oocytes recruited after follicular phase and luteal phase stimulations in the same ovarian cycle were similar. Our data indicate that LPS is not correlated with a higher aneuploidy rate.

Limitations, reasons for caution: These findings are based on a small sample size of patients in a short period. More embryological and clinical data is required to confirm the benefits of DuoStim, as well as an analysis of its cost-effectiveness.

Wider implications of the findings: Many strategies have been proposed to manage poor responder patients, however, a consensus upon which is the most beneficial has not been yet reached. Double estimulation. In selected patients with low oocyte yield, DuoStim should be proposed for oocyte or embryo accumulation considering the cost-efficiency — especially in older women.

Trial registration number: not applicable

P-722 Prevalence of hyperprolactinaemia in subfertile ovulatory women and its impact on fertility treatment outcome

M. Wojcik[1], S. Amer[2], K. Jayaprakasan[3]

[1]University of Nottingham, Medicine, Nottingham, United Kingdom ; [2]Royal Derby Hospital, Gynaecology, Derby, United Kingdom ; [3]Royal Derby Hospital, Fertility Unit, Derby, United Kingdom

Study question: The objectives were to establish the prevalence of hyperprolactinaemia in subfertile ovulatory women in comparison to oligo-anovulatory women, and to evaluate whether hyperprolactinaemia influences fertility treatment outcome.

Summary answer: Prevalence of hyperprolactinaemia was 27.3% in subfertile ovulatory women, with most (86.5%) having mildly raised prolactin. Hyperprolactinaemia did not influence fertility treatment outcome.

What is known already: Prolactin has been linked to ovulation and fertility. Prolactin testing is not generally recommended for subfertile women with regular menstrual cycles, which is a surrogate marker of ovulation. However, some clinicians, particularly in general practice, still perform prolactin testing as part of baseline endocrine profile. Finding of subtle hyperprolactinemia in those women is not uncommon and this poses a dilemma whether such finding will need further evaluation. Furthermore, its impact on fertility treatment outcome is not known.

Study design, size, duration: This observational study included review of electronic hospital database of all women (n=1010), who attended a secondary level fertility clinic over a four-year period between 2015 and 2019. Data of prospectively recorded data was collected retrospectively for analysis.

Participants/materials, setting, methods: All women referred to the clinic with records of endocrine measurements were included. Women with incomplete data and patients who were already on treatment for hyperprolactinaemia were excluded. Data was collected from the hospital electronic database. Endocrine levels, clinical pregnancy outcome, and pituitary MRI, if performed, were recorded on an encrypted excel spreadsheet. Patients were identified as hyperprolactinaemic according to the hospital's norm (>496 mIU/L). Data was subsequently analysed using Chi-square Test and Risk Estimates.

Main results and the role of chance: 1010 women attended the fertility clinic during the studied period, and data from 835 women was included in the analysis. 597 women (71.5%) were in the ovulatory group and 238 (28.5%) were in the oligo-anovulatory group. Surprisingly, prevalence of hyperprolactinaemia was higher in the ovulatory women than in the oligo-anovulatory women (27.3% vs 15.5%; OR: 2.1; 95%CI: 1.4-3.1). Further investigation into the severity of hyperprolactinaemia distinguished three hyperprolactinaemia groups: mild (496-1000mIU/L), moderate (1000 – 2000mIU/L) and severe (>2000mIU/L). In the ovulatory group, mild hyperprolactinaemia was found in 141 patients (23.6%), moderate hyperprolactinaemia in 21 patients (3.5%), and severe hyperprolactinaemia in 1 patient (0.2%). In oligo-anovulatory patients, 31 (13.0%) had mild hyperprolactinaemia, 4 (1.7%) had moderate hyperprolactinaemia, and 2 (0.8%) had severe hyperprolactinaemia. Rates of clinical pregnancy (either natural or following treatment) were similar between hyperprolactinaemic and normoprolactinaemic ovulatory women.

Limitations, reasons for caution: This study was of a retrospective nature and some of the data was missing, however both of these factors would have created a minimal bias, considering the large sample size.

Wider implications of the findings: There is a high prevalence of hyperprolactinaemia among ovulatory women, however most had mildly raised levels, which may not be clinically relevant. Prolactin is responsive to minimal stress, and high levels do not influence pregnancy outcome. Prolactin measurement is not needed in regularly menstruating women, and normal ranges need redefining.

Trial registration number: Not applicable

POSTER VIEWING SESSION

REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY

P-723 Cumulative live-birth rate over multiple cycles of in vitro fertilization in Chinese advanced-age women over 35 years old

F. Gu[1], S. Ruan[1], C. Luo[1], C. Zhou[1]

[1]The First Affiliated Hospital of Sun Yatsen university, Center for Reproductive Medicine, Guangzhou, China

Study question: How does age and ovarian response influence cumulative live birth rates (CLBRs) over multiple IVF/ICSI cycles in Chinese advanced maternal age (AMA) women?

Summary answer: The optimal and conservative CLBRs over 4 complete IVF/ICSI cycles for women over 35 years were 58.0% and 41.7%, varied by age and ovarian response.

What is known already: Traditionally, IVF success rate has been reported in terms of live birth after a single fresh embryo transfer. However, with the international shift towards single embryo transfer and cryopreservation of surplus embryos, CLBR encompassing live delivery outcomes from the fresh and all subsequent frozen embryo transfer (FET) over several complete IVF cycles is a more critical outcome. Age and ovarian response have been demonstrated to be associated with the likelihood of CLBR over multiple IVF/ICSI cycles, while how different ovarian response according to POSEIDON criteria influences the outcome within the same female age has seldom been properly investigated.

Study design, size, duration: This observational cohort study was carried out in a university-affiliated tertiary centre, including 4102 Chinese women aged ≥35 years, who initiated IVF/ICSI treatment from 1st January 2009 to 30th November 2015 and were followed until 31st May 2017. Cycles for PGT, embryo storage or with severe data missing were excluded. Overall, 3486 Chinese advanced age women undergoing 5088 IVF/ICSI cycles were included in the analysis.

Participants/materials, setting, methods: All participants were categorized into four age group: 35-37yrs, 38-39yrs, 40-42yrs and ≥43 yrs. Patients were then categorized into subgroups of non-low prognosis, POSEIDON group 2 and POSEIDON group 4 based on the ovarian response. The primary outcome was the CLBRs across all cycles in different age groups. The secondary outcome was to analyze the CLBRs in the same age group but with different ovarian response. Optimal, and conservative CLBRs were estimated.

Main results and the role of chance: The optimal and conservative estimated CLBRs over 4 IVF/ICSI treatment cycles of Chinese women aged ≥35 years were 58.0% and 41.7%, respectively. The optimal and conservative estimated CLBRs up to the fourth cycle decreased from 71.7% and 49.8% for women aged 35-37yrs (n= 2447 cycles) to 62.0% and 42.4% for women aged 38-39 years (n=1185cycles), 40.3% and 26.4% for women aged 40-42 years (n=1023 cycles) and 7.7% and 5.5% for women aged ≥43 years (n=431 cycles). In all the age groups, the LBRs of first cycle in the non-low prognosis group were higher than in the POSEIDON group 2[unexpected poor ovarian responder(POR)], followed by the POSEIDON group 4(expected poor responder). Fortunately, younger (<40 years old) unexpected POR had a 4-cycle CLBR of ~57.3% to ~70% which were comparable to those with non-low prognosis patients with a CLBR of ~74.5% to ~81%. Yet the 4-cycle CLBR of older unexpected (≥40

years old) POR was ~39.5% which was 50% lower than those with adequate ovarian reserve. The younger expected POR had a higher 4-cycle CLBR rate with 46.1%, while the CLBR was only 21.5% in the older expected POR.
Limitations, reasons for caution: High withdrawal rate due to poor prognosis of the AMA patients reduced the precision of the estimates.
Wider implications of the findings: This is the first relevant data of the CLBRs over multiple IVF cycles in Chinese women, which vary between age and ovarian response. Patients <43 years could benefit from extending IVF cycles up to three or four, whlie those over 43 should be discouraged from IVF cycles with autologous oocytes.
Trial registration number: not applicable

P-724 Paternal and Maternal Preconception Exposure to Phenol and Phthalate Mixtures in Relation to Preterm Birth Risk

V. Mustieles[1], Y. Zhang[1], P.L. Williams[2], B.J. Wylie[3], A.M. Calafat[4], R. Hauser[1], I. Souter[5], C. Messerlian[1]

[1]Harvard T.H. Chan School of Public Health, Environmental Health, Boston, U.S.A. ; [2]Harvard T.H. Chan School of Public Health, Biostatistics, Boston, U.S.A. ; [3]Beth Israel Deaconess Medical Center, Obstetrics and Gynecology, Boston, U.S.A. ; [4]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, U.S.A. ; [5]Massachusetts General Hospital, Obstetrics and Gynecology, Boston, U.S.A.

Study question: To what extent do paternal and maternal preconception phenol and phthalate chemical mixtures interact and contribute to preterm birth risk?
Summary answer: Couples' preconception exposure to chemical mixtures was associated with an increased risk of preterm birth, with a complex interplay between both parents.
What is known already: Humans are continuously exposed to low-doses of multiple endocrine disrupting chemicals (EDCs) and mounting evidence of heightened toxicity from mixtures exists. While maternal and paternal preconception exposures to select phenols and phthalates have been associated with increased preterm birth risk in single-chemical analyses, their joint effect in the context of complex mixtures is unknown. Emerging data highlight the potential for EDCs to exert enduring epigenetic modifications in male and female gametes, a potential mechanism related to adverse reproductive outcomes. The preconception period is thus increasingly recognized as a vulnerable window for environmental perturbation.
Study design, size, duration: Ongoing prospective preconception cohort of subfertile couples recruited between 2005 and 2018 in a large fertility center in Boston, Massachusetts, USA. A total of 384 singletons were included in the current analysis (384 mothers, 211 fathers, 203 couples) from the Environment and Reproductive Health Study. Gestational age was abstracted from medical records and validated using clinical guidelines for births following medically assisted reproduction. Preterm birth was defined as live births <37 weeks gestation.
Participants/materials, setting, methods: We used principal component analysis (PCA) with Poisson regression, and Probit Bayesian Kernel Machine Regression (BKMR) to examine maternal and paternal mean preconception urinary concentrations of EDC mixtures on preterm birth risk. Log-concentrations of bisphenol A (BPA), parabens, and eleven phthalate metabolites including those of di(2-ethylhexyl) phthalate (DEHP) were included in both maternal and paternal mixture models. Hierarchical variable selection was fit for couple-based BKMR models by dividing mixtures into maternal *vs.* paternal groups.
Main results and the role of chance: The mean (SD) gestational age among singletons was 39.3 (1.7) weeks with 8% born preterm. PCA identified four main factors, with loading scores for the DEHP-BPA factor showing associations with an increased preterm birth risk in both mothers (adjusted Risk Ratio (aRR): 1.36, 95%CI: 1.00, 1.84) and fathers (aRR: 1.47, 95%CI: 0.90, 2.42). Separate maternal and paternal BKMR models identified maternal preconception BPA and paternal preconception mono(2-ethylhexyl) phthalate concentrations were positively associated with preterm birth when the remaining mixture components were held at their median concentrations. In analyses restricted to couples, BKMR models with hierarchical variable selection showed a similar relative contribution of both paternal [Posterior Inclusion Probability (PIP): 61%] and maternal (PIP: 77%) preconception mixtures on preterm birth. Additionally, a positive cumulative joint effect of couples' preconception mixtures assessed through increasing mixture quantiles on preterm birth was also observed.
Limitations, reasons for caution: Although our mixture analysis extended information provided by previous single-chemical models, these findings may not be generalizable to fertile couples. While human populations are normally exposed to dozens of chemical families, our mixtures analyses focused on non-persistent EDCs such as those used in plastics (BPA, DEHP) and in cosmetics (parabens).
Wider implications of the findings: This study suggests a complex interplay between paternal and maternal exposures to mixtures of non-persistent chemicals, with both windows of exposure jointly and cumulatively contributing to the risk of preterm birth.
Trial registration number: Not Applicable

P-725 Refitting and adapting the van Loendersloot prognostic model for In Vitro Fertilization

J. Devroe[1], K. Peeraer[1], G. Verbeke[2], C. Spiessens[1], J. Vriens[3], E. Dancet[1]

[1]Leuven University Hospital, Gynaecology, Leuven, Belgium ; [2]Leuven Biostatistics and statistical Bioinformatics Centre, Public Health and Primary Care- KU Leuven, Leuven, Belgium ; [3]Laboratory of Endometrium- Endometriosis & Reproductive Medicine, Development and Regeneration- KU Leuven, Leuven, Belgium

Study question: What is the performance of the refitted (using previous cycle variables) and adapted (using current cycle variables) van Loendersloot prognostic model for our clinic's IVF?
Summary answer: The Refitted and the Adapted van Loendersloot model are both valuable for predicting the chance on a live birth after one complete IVF cycle.
What is known already: Prognostic models combine clinical and/or IVF-laboratory variables with different weights to predict IVF-success. The impact of informing couples about their IVF-prognosis on their expected success rates, distress and IVF-discontinuation has yet to be studied. The 'van Loendersloot model', including previous cycle IVF-laboratory variables, proved performant for two clinics and is relevant for informing couples prior to starting another cycle (Sarais, et al., 2016, van Loendersloot, et al., 2013). This model could potentially include current cycle IVF-laboratory variables, relevant for informing couples at the time of embryo transfer.
Study design, size, duration: We retrospectively studied a cohort from our clinic of 1281 IVF cycles of 591 couples (period 2010-2018). We assessed the performance of the Refitted van Loendersloot model including previous cycle IVF-laboratory variables and of the Adapted van Loendersloot model including current cycle IVF-laboratory variables.
Participants/materials, setting, methods: Eligible couples completed at least one IVF-cycle with own fresh gametes after a previous IVF-cycle with the same partner in our clinic. Binary logistic regression analysis with cumulative live birth as dependent variable identified intercept and relative weights of its variables. Performance was expressed in terms of discrimination (i.e. c-statistics) and calibration (i.e. Hosmer-Lemeshow goodness-of-fit, calibration model, Nagelkerke R^2, comparison of five disjoint groups formed by the quintiles of the IVF-prognoses and a calibration plot).
Main results and the role of chance: A total of 344 live births were obtained over 1281 oocyte aspirations (26.9%). The Refitted and Adapted van Loendersloot models showed a c-statistic respectively 0.68 (95% CI: 0.65-0.71) and 0.74 (95% CI: 0.70-0.77). The Refitted model showed no significant miscalibration (p=0.793), a good calibration model (intercept=0.00; 95%CI: -0.23–0.23 and slope=1.00; 95%CI: 0.79-1.21), good prognosis to observed ratios (0.9-1.0) and a good calibration plot. The Adapted model showed no significant miscalibration (p=0.093), a good calibration model (intercept=0.00; 95% CI: -0.18–0.18; slope=1.00; 95%CI: 0.83-1.17), good prognosis to observed ratios (0.9-1.1) and its calibration plot was even better than that of the refitted model. The calibration plots mainly differed regarding the overlap between the 95% confidence intervals of the mean observed live birth rate of the disjoint groups next to each (i.e. 4/4 for the Refitted and 1/4 for the Adapted model). These results confirm the robustness of the van Loendersloot prognostic model in both the refitted version, which can be calculated prior to an IVF-cycle, and in

the novel adapted version, which can be calculated at the time of embryo transfer.
Limitations, reasons for caution: We conducted a complete case analysis and studied an 8-year retrospective period, over which IVF-protocols changed slightly.
Wider implications of the findings: The performance of the Refitted and the Adapted van Loendersloot model in our clinic allows informing couples on their IVF-prognosis prior to an IVF-cycle or during an embryo transfer and studying the impact on couple's expected success rates, distress and IVF-discontinuation.
Trial registration number: Not applicable

P-726 The intimate vaginal hygiene practice correlates with the vaginal microbiota; an observational study of 328 in vitro fertilization (IVF) patients.

K. Rosenstand Jensen[1], P. Humaidan[1], H.O. Elbæk[1], B. Alsbjerg[1], R.J. Laursen[1], A. Mikkelsen[1], M.B. Jensen[1], L. Prætorius[2], N.L.C. Freiesleben[2], A. Pinborg[3], V. Hartvig[4], J.S. Jensen[5], T. Haahr[1]

[1]Regional Hospital Skive - Skive - Denmark -, The Fertility Clinic, Skive, Denmark ; [2]Amager-Hvidovre Hospital, Department of Gynecology and Obstetrics, Hvidovre, Denmark ; [3]Rigshospitalet, Department of Gynecology and Obstetrics, Copenhagen, Denmark ; [4]VivaNeo Stork Fertility Clinic, Copenhagen, Copenhagen, Denmark ; [5]Statens Serum Institut, Copenhagen, Copenhagen, Denmark

Study question: What is the intimate hygiene practice of women in IVF treatment and does it correlate with the vaginal microbiota?
Summary answer: Overall, 34% had an abnormal vaginal microbiota (AVM) and a total of 23% practiced vaginal douching. Of douching women, 47% had AVM.
What is known already: AVM is a molecularly defined vaginal dysbiosis dominated by anaerobic bacteria such as *Gardnerella vaginalis*. In contrast, vaginal eubiosis is considered dominated by one or more *Lactobacillus spp*. Recent independent studies have shown that AVM is significantly correlated with poor reproductive outcomes in IVF patients. Although the definitive etiology of AVM is still debated, intimate vaginal hygiene practices have been suggested as a possible etiological factor. Currently the knowledge about this issue is limited and in this study in IVF patients, we explored the impact of different vaginal hygiene practices on the presence of AVM.
Study design, size, duration: Observational cross-sectional study, including a total of 328 IVF patients from four Danish fertility centers. Patients were included from March 2019 to January 2020.
Participants/materials, setting, methods: IVF patients were eligible for inclusion if they underwent their first, second or third IVF cycle either as heterosexual couples, singles or lesbians. Besides standard gynecological work-up, patients reported intimate hygiene practices (soap, menstrual protection, douching, probiotics) in a questionnaire. Vaginal swabs were obtained prior to ovarian stimulation and subsequently subjected to quantitative PCR testing, targeting DNA of AVM bacteria.
Main results and the role of chance: From the population of 328 women, a total of 34% (112/328) were diagnosed with AVM. The largest proportion of women used water, only, (42%; 139/328) for intimate hygiene, whereas 38% (124/328) used intimate soaps of low pH and 28% (92/328) used regular soaps. Only 3% of the women (9/328) used vaginal probiotics. Interestingly, 23% (75/328) of the women reported vaginal douching, which significantly correlated with the presence of AVM (p 0.01). None of the remaining hygiene methods showed any correlation with the vaginal microbiota.

The largest proportion of women used pads for menstrual protection (42%; 137/328), followed by the use of tampons, alone (24%; 80/328) or a combination hereof (22%; 71/328). 11% used a menstrual cup (37/328). Only 1% reported use of other unspecified menstrual protection. The use of a menstrual cup significantly correlated with a normal vaginal microbiota ($p < 0.01$).

Furthermore, active smoking or the consummation of >7 units of alcohol per week significantly correlated with AVM (p 0.03 and $p < 0.01$, respectively). Finally, a fishy vaginal odor was significantly more frequently reported in AVM positive patients; 10% (11/112) in the AVM group as compared to 4% (9/216) in the normal group (p 0.04).
Limitations, reasons for caution: To the best of our knowledge, this is the first large study investigating the intimate hygiene practices and its correlation to AVM in an IVF population. As these results describe the practices of a Scandinavian IVF population, results may differ from other settings and ethnicities.
Wider implications of the findings: Exploration of the cause-and-effect relations between intimate hygiene habits and AVM is needed, requiring intervention-based prospective studies. This will lead to evidence-based advice on intimate hygiene and AVM prevention possibly increasing live birth rates in infertile patients. The high prevalence of douching in a Danish IVF population needs further investigation.
Trial registration number: NCT03420859

P-727 The InfertilScore, a couple-based machine learning model in patients with idiopathic infertility.

G. BACHELOT[1], R. Lévy[1], C. Faure[1], S. Czernichow[2], C. Dupont[1], A. Lamazière[3]

[1]Sorbonne Université- Saint Antoine Research center, INSERM équipe Lipodystrophies génétiques et acquises, PARIS, France ; [2]APHP- Hôpital européen Georges-Pompidou, Service de nutrition, Paris, France ; [3]Sorbonne Université- Saint Antoine Research center, INSERM équipe Microbiote-intestin et inflammation, Paris, France

Study question: Could unexplained infertility be diagnosed thanks to determined anthropometric, antioxidative and metabolic signature modeled using machine learning?
Summary answer: We designed a supervised multivariate model which stratify subfertile couples thanks to their anthropometric, antioxydative and metabolic signatures.
What is known already: The efficacy of traditional clinical approaches for unexplained infertility lack objective criteria to guide the medical decision for a non-surgical fertility treatment or/and assisted reproductive therapy (ART). Even if, by definition, no cause is clearly identified, the environment and lifestyle should be also considered as idiopathic infertility origins. Based on previous studies, we observed that sedentary behavior, physical inactivity, body composition and metabolic status were closely in relation to idiopathic infertility. Consequently, an algorithm on modifiable risk factors is therefore strongly needed for the management and follow-up of idiopathic infertility.
Study design, size, duration: The data of this study concerned infertile (n=96) and fertile (n=100) couples who have been recruited in the ALIFERT cross-sectional case-control study between September 2009 and December 2013 (N° P071224). The ALIFERT study has been designed to assess the association between lifestyle factors and idiopathic infertility in couples (National biomedical research Id. P071224, ethics committee approval ("Comité de Protection des Personnes") AOM 2009-A00256-51, NEudra CT 08180, clinicaltrials.gov NCT01093378).
Participants/materials, setting, methods: An accurate machine learning model was sequentially designed among 80 to 13 anthropometric, antioxidative and metabolic selected features from the ALIFERT patients.
Main results and the role of chance: Based on the ALIFERT cohort, our results showed that i) couples modeling approach was more discriminant than a model in which men and women parameters are considered separately ii) the most important variables for the projection were related to anthropometric and metabolic disorders and oxidative status iii) after reduction of our system dimensions (from 80 to 13 variables) we proposed a new algorithm to stratify fertile *vs* subfertile patients. Eventually, in order to use this tool to help the therapeutic decision, we put into perspective the InfertilScore model and the successes and failures to get pregnant after a year post ART (assisted reproductive technology). We did find some relationships between the treatment intensity (*in vitro* fertilization (IVF), artificial insemination) and the severity of idiopathic couple infertility based on our InfertilScore model. Our conclusions will be presented at ESHRE congress.
Limitations, reasons for caution: Despite a multicentric approach, these results showed a need to test the prediction strength of the InfertilScore on a future independent replication cohort.
Wider implications of the findings: In the complex situations of couples for whom, the result of the standard fertility assessment has not made it possible to highlight a clear cause of infertility, the InfertilityScore could be considered as

a useful tool for therapeutic decision-making and medical guidance in reproductive medicine.
Trial registration number: P071224

P-728 Use of Cross Border Reproductive Care in Infertile Women in Hong Kong

D. Ng[1], M.W. Lui[2], S.F. Lai[3], H.Y. Wong[4], T.S.M. Law[5], G.C.Y. Wong[6], E.H.Y. Ng[2]

[1]Pamela Youde Nethersole Eastern Hospital, Department of Obstetrics and Gynaecology, Edinburgh, United Kingdom ;
[2]Queen Mary Hospital, Department of Obstetrics and Gynaecology, Hong Kong, Hong Kong ;
[3]Kwong Wah Hospital, Department of Obstetrics and Gynaecology, Hong Kong, Hong Kong ;
[4]Princess Margaret Hospital, Department of Obstetrics and Gynaecology, Hong Kong, Hong Kong ;
[5]Prince of Wales Hospital, Department of Obstetrics and Gynaecology, Hong Kong, Hong Kong ;
[6]Family Planning Association, Family Planning Association, Hong Kong, Hong Kong

Study question: What are the incidence, demographic characteristics, attitudes and motivations of cross border reproductive care (CBRC) among women with infertility in Hong Kong?
Summary answer: Main reason for CRBC was inadequate accessibility to infertility care; Waiting lists were too long in public and the costs in private were too high.
What is known already: Cross border reproductive care (CBRC) is an escalating global trend where patients travel out of their home country for fertility treatment. It was estimated that around 5% of the fertility care involved CBRC. The reasons for CBRC in Europe included: to avoid legal restrictions at home, to avoid long waiting-lists, for less expensive treatment or the wish for donor anonymity in the context of gamete donation. There are potential advantages and disadvantages of CBRC. Safety concerns are mainly regarding the number of embryos transferred, and thus the risk of multiple pregnancies and the potential exploitation of gamete donors and surrogates.
Study design, size, duration: From 1 Feb 2017- 31 Dec 2019, a cross sectional questionnaire study was conducted in six infertility clinics in Hong Kong. A total of 1,204 questionnaires were collected and analysed.
Participants/materials, setting, methods: A questionnaire was designed to evaluate the use, attitudes and motivations of CBRC in infertile women in Hong Kong. The content of the questionnaire focused on three areas: [1] demographic information, [2] reproductive history and attitudes to fertility, [3] motivations and perspectives on CBRC. Participants completed an anonymous questionnaire, which was distributed by the clinic nurse when they attended the infertility clinic. Participation was entirely voluntary and the questionnaire took approximately 20 minutes to complete.
Main results and the role of chance: A total of 1,204 questionnaires were included. 14.7% of the respondents had used CBRC and 30.9% had plans for it or would consider it. The two main motivational factors were a long waiting time in the public sector and high treatment costs in the private sector, contributing 80.9% and 12.0% of those who had used or would consider CBRC respectively. Only 0.5% of them was motivated for CBRC for law evasion. Majority of our patients chose Taiwan as the preferred destination (69.6%); followed by mainland China (25.8%). Over half of them(55.6%) accessed information from the internet. At the point of data collection, among the patients who had used CBRC, 1.7% suffered from ovarian hyperstimulation syndrome whereas another 1.7% suffered from other types of complications. 70.2% patients believed that the authorities in Hong Kong should formulate some regulations or guidance regarding CBRC. For those participants who had used CBRC or had plans for it, 61.8% indicated that they had received local pretreatment counseling from their home country to assist them with their treatment. 70.2% patients believed that the authorities in Hong Kong should formulate some regulations or guidance regarding CBRC.
Limitations, reasons for caution: We recruited patients from infertility clinic which has a certain degree of bias. Those who had pregnancy following CBRC, single women, single men and same sex couples were not included. Furthermore, the infertility centres in our study cannot be considered as representative of all infertility centres in Hong Kong.
Wider implications of the findings: Based on the results of this study, it can be hypothesized that this CBRC trend will continue until the local health authorities can implement more effective measures to shorten the long waiting list in the public sector. Meanwhile, local reproductive care organizations should establish good practice guidelines on CBRC.
Trial registration number: not applicable

P-729 Country-specific preparation practices for the insertion of intrauterine devices: insertion procedures reported by Health Care Providers in the EURAS-LCS12 study

C. Hagemann[1], A. Bauerfeind[2], K. Heinemann[3]

[1]Berlin Center for Epidemiology and Health Research, Project Management, Berlin, Germany ;
[2]Berlin Center for Epidemiology and Health Research, Statistics, Berlin, Germany ;
[3]Berlin Center for Epidemiology and Health Research, Principal Investigator, Berlin, Germany

Study question: To identify insertion related procedures reported in the "European Active Surveillance Study on LCS12" (EURAS-LCS12) among users of intrauterine device (IUDs) in ten European countries.
Summary answer: Differences regarding insertion preparation were observed in the participating countries. They are not only related to age or parity, but also country-specific practices are relevant.
What is known already: Intrauterine contraceptive methods, such as Mirena and CU-IUDs, have a high contraceptive efficacy and are commonly used. However, the insertion procedure can be painful, and there are different procedures available to make the insertion less unpleasant, such as pain medication.
Study design, size, duration: Large, prospective, controlled, non-interventional, long-term cohort study with active surveillance of approximately 65,000 study participants in ten European countries: Austria, Czech Republic, Finland, France, Germany, UK, Poland, Spain, Italy, and Sweden. Women are enrolled by their Health Care Provider (HCP) after the IUD insertion. The study started in 2014 and the final study report is planned for 2025.
Participants/materials, setting, methods: In the EURAS-LCS12 study, a network of prescribing HCPs is enrolling women with a newly inserted IUD. HCPs and participating women complete a baseline questionnaire including information on insertion related procedures and issues. Patients receive 5 follow-up questionnaires within 3 years. All patient-reported outcomes of interest are validated with the treating physician.
Main results and the role of chance: Until end of August 2019, 55,793 women were enrolled, thereof 5,988 (11%) Jaydess, 26,183 (47%) Mirena, 3,671 (7%) Kyleena and 18,840 (34%) CU-IUD users. Users of the smaller hormonal IUDs were considerably younger (Jaydess: mean age 26,9 years, Kyleena 27.8 y) compared to Mirena (35.9 y), and 77% of Jaydess and 54% of Kyleena users were nulliparous compared to 15% of Mirena users. Pharmacological preparation (e.g. prostaglandins, painkiller) were used in 48% of Jaydess, 53% of Kyleena, 26% of Mirena and 31% of CU-IUD users, however, large differences were observed in the different countries, ranging from 7% in UK to 67% in Austria. Prostaglandins were most frequently used in Austria (44%) and Germany (33%), and painkillers in Sweden (57%), Austria (38%) and Finland (30%). This is also related to parity: of nulli-parous women, 20% received prostaglandins and 32% painkillers, compared with 9% prostaglandins and 13% painkiller used in parous women.

Use of general anesthesia was low in all countries (1 to 4%), and local anesthesia was most frequently used in the UK (43%) and Austria (31%), and less often in the other countries: Czechia (1%), Spain (2%), Finland and Italy (3%), France (6%), Poland (7%), Germany (10%) and Sweden (11%).
Limitations, reasons for caution: Age distribution differed largely among the study cohorts, and thus, also the proportion of parous women. In addition, different standard practices regarding insertion of the IUD were seen in the different countries.
Wider implications of the findings: IUDs are a comfortable contraceptive method which don't require daily intake by women. However, the insertion procedure might be unpleasant, especially for nulli-parous women. In the participating countries, different methods for preparation of the insertion were seen. Further research is necessary to assess the impact of different methods.
Trial registration number: NCT02146950

P-730 High prevalence of Mycoplasma /Ureaplasma in asymptomatic oocyte donors

P.L. Aldama González[1], M. Lacarcel[1]

[1]Eva Fertility Clinics, EvaBank, Madrid, Spain

Study question: What is the current prevalence of Sexually Transmitted Infections (STIs) in asymptomatic oocyte donors?
Summary answer: The current prevalence of STIs in asymptomatic oocyte donors is higher than expected, especially for Mycoplasma and Ureaplasma Urealyticum.
What is known already: Sexually transmitted infections (STIs) are related to infertility worldwide. The impact of STIs such as Mycoplasma hominis, Ureaplasma Urealyticum and Chlamydia Trachomatis on fertility has been debated for several decades without being able to reach definitive conclusions due to isolating difficulties and absence of symptoms.
Study design, size, duration: This is a retrospective observational study including all the asymptomatic oocyte donors with complete STIs detection report between July and December of 2019.
Participants/materials, setting, methods: A total of 72 donors (18 to 29 years old) were included in the study. Samples from each donors were cultured for Mycoplasma Hominis, Ureaplasma Urealyticum and Ureaplasma Parvum. Chlamidya Tracomatis antigen and Neisseria Gonorrhoeae antigen in cervical sample, Pallidum Antitreponema and Herpes 1/2 antibodies were analyzed. General vaginal microbiological culture was performed also for each donor. The statistical analysis was performed using measures of central tendency, and Pearson's Chi2, using the SPSS version 25
Main results and the role of chance: STIs were found in 58% of asymptomatic oocyte donors (n =42). Therewas no correlation between STIs with age, race, occupation, or marital status. However, STIs were significantly associated with low education (p=0.045). Mycoplasma hominis was founded in 39%of the cases (n=16), Ureaplasma Urealyticum in 56% (n=23), Ureaplasma Parvum 14% (n=6). Chlamydia Trachomatis 9% (n=4). Mycoplasma hominis and Ureaplasma Urealyticum was founded together in 24% (n=10), and Ureaplasma spp in 14% (n=6).
Limitations, reasons for caution: This is an small observational study based on a retrospective data analysis. Better extrapolation of the results could be validated by performing an extensive prospective study.
Wider implications of the findings: The young healthy population must be targeted with preventive educational programs. Although STI is not investigated routinely but in long run, it can might have negative impact on fertility rate in society.
Trial registration number: Not Applicable

P-731 The impact of body mass index on in vitro fertilization performance in infertile women

A. Albu[1], D. Albu[2]

[1]Carol Davila University of Medicine and Pharmacy, Endocrinology, Voluntari, Romania ;
[2]Carol Davila University of Medicine and Pharmacy, Obstetrics and Gynecology, Voluntari, Romania

Study question: Which is the relationship between the entire spectrum of body mass index (BMI) and in vitro fertilization (IVF) parameters?
Summary answer: Both low BMI and overweight BMI seem to be associated with oocytes and zygotes number and the responder type.
What is known already: The association between BMI and IVF outcome is widely debated, the results of the available studies being divergent. While higher BMI seems to have a negative impact on reproductive outcome, the data regarding the connection between low BMI and reproductive parameters are limited.
Study design, size, duration: We performed a retrospective study in the Department of Reproductive Medicine of a private hospital. The medical records of all consecutive patients who underwent IVF between January 2015 and December 2018 with all causes of infertility were reviewed.
Participants/materials, setting, methods: One thousand three hundred thirty-seven patients were included in the study (mean age 34.7±4.2 years). Patients were divided according to BMI in underweight (n=116, BMI<18.5kg/sqm), normal weight (n=971, BMI 18.5-24.99 kg/sqm), overweight (n=195, BMI 25-29.99 kg/sqm) and obese (n=55, BMI>30 kg/sqm). The number of oocytes obtained at egg collection and the zygotes number were recorded. Patients were also categorized according to the type of responder: poor responder, low responder, normal responder and hyper responder.
Main results and the role of chance: BMI was positively correlated with age (rho = 0.141, p < 0.0001) and negatively with oocytes (rho = - 0.068, p = 0.014) and zygotes number (rho = - 0.080, p = 0.004). In a multivariate regression model, after adjustment for age and AMH serum level, being underweight was positively associated with oocytes number (beta = 0.072, p = 0.002) and with zygotes number (beta = 0.059, p = 0.02) and being overweight was negatively associated with oocytes number (beta = - 0.051, p = 0.028). In a multinomial regression model, after adjustment for age and AMH serum level, underweight patients had an increased chance of being normal responder in comparison with normal weight patients (OR 1.4, CI 1.2-2), while overweight patients had decreased odds of being normal responder in comparison with normal weight patients (OR 0.56, CI 0.29-0.8)
Limitations, reasons for caution: The main limitation of the study is the relatively small number of patients included in the obese and underweight category, probably affecting the statistical analysis.
Wider implications of the findings: Our study showed the association between low and high BMI and oocytes and zygotes number, which were reported to be significant predictors of live birth, therefore offering possible ways to influence IVF outcome by modifying body weight.
Trial registration number: NA

P-732 Cumulative live birth rates among gestational carriers in altruistic surrogacy arrangements

J. Attawet[1], A. Wang[1], Z. Li[1], K. Hammarberg[2], L. Johnson[3], E. Sullivan[4]

[1]University of Technology Sydney, Public Health, Sydney, Australia ;
[2]Monash University, Public Health, Melbourne, Australia ;
[3]Victoria Assisted Reproductive Treatment Authority, varta, Melbourne, Australia ;
[4]University of Newcastle, Health and Medicine, Callaghan, Australia

Study question: What is the cumulative live birth rate (CLBR) among gestational carriers in altruistic surrogacy arrangements?
Summary answer: The CLBR among gestational carriers ranged from 23.5% after the first embryo transfer cycle to 50.6% after six consecutive embryo transfer cycles.
What is known already: CLBR is a measure of the success of assisted reproductive technology (ART) which demonstrates how each additional treatment adds to the chance of having a live birth. It is commonly used in the context of autologous treatment where women have used their own eggs.
Study design, size, duration: This was a population-based retrospective cohort study of all intended parents and gestational carriers who had at least one embryo transfer cycle in the state of Victoria, Australia between 2009 and 2016. Pregnancy and birth outcomes were followed until a live birth was achieved or until June 30, 2017, whichever came first.
Participants/materials, setting, methods: Data collected by the Victorian Assisted Reproductive Treatment Authority (VARTA) on all gestational surrogacy arrangements in Victoria between 2009 and 2017 were analysed. The primary outcome was cumulative live birth, which was defined as live deliveries with at least one live born baby resulting from initiated stimulated cycles and associated thaw cycles. Life-table was used to calculate the CLBR.
Main results and the role of chance: There were 66 intended parents and 81 gestational carriers. Of the 170 embryo transfer cycles, the majority were single embryo transfers (97.1%), using frozen/thawed embryos (97.6%) which had been fertilized by intracytoplasmic sperm injection (ICSI) (77.6%). The cumulative live birth rate was 23.5% (95% CI, 15.6-33.8%) after the first cycle and increased to 50.6% (95% CI, 40.0-61.2%) after the sixth cycle. Of the 41 deliveries, 40 were singletons and one was a twin delivery. Two of the 42 babies were preterm, two were low birthweight and one was small for gestational age.
Limitations, reasons for caution: Although this population-based study included all gestational surrogacy arrangement in Victoria, the sample size is small. The study was conducted in a setting where only altruistic surrogacy is legal, and the findings may not be generalizable to settings where commercial surrogacy is undertaken. .

Wider implications of the findings: Altruistic surrogacy arrangement is unlikely to reduce the cumulative live birth rate. Among gestational carriers, surrogacy treatment can be offered up to six consecutive embryo transfer cycles without reducing the chance of a live birth. This estimate can be used in counselling and decision-making for all parties involved in surrogacy.
Trial registration number: not applicable

P-733 Childless by circumstance – the fertility experiences of women who wanted children

J. Harper[1], D. Chauhan[1], E. Jackson[2]

[1]Institute for Women's Health, EGA Institute for Women's Health, London, United Kingdom ;
[2]London School of Economics, Law, London, United Kingdom

Study question: What reproductive experiences did childless women go through and how do they feel about being childless?
Summary answer: Women experienced social infertility, miscarriages, and if they went through IVF, they mainly stopped for emotional reasons. The majority felt negatively about being childless.
What is known already: Childlessness is increasing across many middle- and high-income countries, for a wide variety of reasons, including increasing gender equality, greater respect for reproductive autonomy, infertility and, in some cases, an inability to find a partner. Many women have postponed childbearing and the age of first birth is increasing annually. Europe has the lowest fertility rate on record, as well as a record number of women being permanently childless.
Study design, size, duration: An online survey study was developed and validated with help from childless women. The survey consisted of questions with quantitative-answer formats as well as open-text qualitative answer formats. It was promoted through social media. The survey remained open online for 15 days.
Participants/materials, setting, methods: The survey was only completed by women who were aged 46 years of age and above, who had wanted children but were childless. In total 303 survey responses were collected, 176 of which were complete surveys. The data was explored through the survey platform's data reporting function. The qualitative data was analysed with a qualitative descriptive analysis approach.
Main results and the role of chance: 16.3% (n=27) of women who wanted children reported that they did not try to have children, most commonly due to the lack of a partner (40%, n=11). Of the women who tried to have children (n=139), 70.5% (n=98) had used calendar-based menstrual cycle tracking methods to identify their fertile window and many had fertility checks including hormone tests (75.5%) and ultrasound scans (71.2%). A significant proportion of women had experienced a miscarriage (38.8%). Many women decided not to have any fertility treatments (43.2%, n=60). For those that did undergo fertility treatments, many had tried in vitro fertilization (42.4% (n=59), and this was mainly privately funded. The most common reason women gave for stopping fertility treatment was due to emotional reasons (42.4%, n=59). When asked how women felt about their childlessness, the most common emotions identified were sadness (53.1%, n=85), gradual acceptance (36.3%, n=58), and ostracism (33%, n=53).
Limitations, reasons for caution: The use of the word childless was discussed. 'Childless by circumstance' was agreed in order not to presuppose reasons for women's childlessness, or to assume that women's attitude towards their childlessness was fixed. 127 participants started but did not complete the survey. The survey was only advertised on social media.
Wider implications of the findings: This study has listened to the self-reported experiences of women who wanted children, but who are childless. Support for unsuccessful fertility patients and other childless women should be expanded, and emphasis on fertility education should be established in order to ensure that women are better informed about their fertility.
Trial registration number: Not applicable

P-734 Cost-effectiveness of medically assisted reproduction or expectant management for unexplained subfertility: when to start treatment?

R. Van Eekelen[1], M.J. Eijkemans[2], M. Mochtar[1], F. Mol[1], B.W. Mol[3], H. Groen[4], M. Van Wely[1]

[1]Amsterdam UMC- Academic Medical Centre, Centre for Reproductive Medicine, Amsterdam, The Netherlands ;
[2]University Medical Centre Utrecht- Julius Centre, Department of Biostatistics and Research Support, Utrecht, The Netherlands ;
[3]Monash Medical Centre, Department of Obstetrics and Gynaecology, Melbourne-Monash, Australia ;
[4]University Medical Centre Groningen, Department of Epidemiology, Groningen, The Netherlands

Study question: Over a time period of three years, which order of expectant management (EM), IUI-OS and IVF is the most cost-effective for couples with unexplained subfertility?
Summary answer: The most cost-effective scenario depended on the monetary value assigned to a live birth: EM-EM-IVF (if assigned <€32,000) or EM-IUIOS-IVF when assigned more.
What is known already: IUI-OS and IVF are commonly used for unexplained subfertility although these couples can still conceive naturally. Few countries have guidelines on when to proceed with medically assisted reproduction (MAR) but there is a lack of evidence to support these strategies. The increased uptake of IUI-OS and IVF over the past decades and costs related to reimbursement of these treatments are pressing concerns to health service providers. For MAR to remain affordable, sustainable and a responsible use of public funds, guidance is needed on the cost-effectiveness of treatment strategies for unexplained subfertility.
Study design, size, duration: We developed a cost-effectiveness model that follows couples with unexplained subfertility for a total of 3 years from diagnosis onwards, divided into three periods of 1 year each. The model was based on contemporary evidence i.e. the dynamic prediction model for natural conception and the network meta-analysis on RCTs in MAR for unexplained subfertility. We changed the ordering of the three options, EM, IUIOS and IVF to yield different treatment scenarios.
Participants/materials, setting, methods: The main outcomes were the probability of live birth and aveage costs. We chose the Dutch societal perspective. The probabilities of live birth after EM were taken from the dynamic prediction model for natural conception. The relative effects of IUI-OS and IVF were taken from the network meta-analysis and applied to probabilities of live birth after EM. We applied discounting. Uncertainty was taken into account using probabilistic sensitivy analyses, replicating the simulation model 20,000 times.
Main results and the role of chance: From IVF-EM-EM to EM-IUIOS-IVF, the probability of live birth varied from approximately 54% to 64% and the average costs from approximately €4000 to €9000. The scenarios IVF-EM-EM and EM-IVF-EM were dominated by EM-EM-IVF as the latter yielded a higher cumulative probability of live birth at a lower cost. The scenario IUIOS-IVF-EM was dominated by EM-IUIOS-IVF as the latter yielded a higher cumulative probability of live birth at a lower cost. After removal of scenarios that were dominated, the incremental cost-effectiveness ratio (ICER) for EM-IUIOS-IVF was approximately €31,000 compared to EM-EM-IVF. The range of ICER values between the lowest 25% and highest 75% of simulation replications was broad.

The net benefit curve showed that when we assume a live birth to be worth approximately €32,000 or less, the scenario EM-EM-IVF had the highest probability to achieve the highest net benefit. When we assume a monetary value per live birth over €32,000, the scenario with the highest probability to achieve the highest net benefit was EM-IUIOS-IVF. Results for subgroups with different baseline prognoses were similar to the primary analysis but yielded different threshold values for the assumed monetary value per live birth.
Limitations, reasons for caution: Our model was at the population level and thus based on average statistics. We also assumed certain model parameters and assessed the influence of these assumptions on our results. The change in relative effectiveness of IVF over time was found to be highly influential on results and their interpretation.
Wider implications of the findings: Two scenarios, EM-EM-IVF and EM-IUIOS-IVF, were the most cost-effective at different monetary values for a live birth with a threshold of €32,000. Our results can be used in determining sustainable MAR protocols for couples with unexplained subfertility that avoids unnecessary treatment.
Trial registration number: Not applicable

P-735 More than 40% of couples leave their supernumerary cryopreserved embryos frozen and destined for discarding

M. Stimpfel[1], B. Valentincic-Gruden[1], J. Kmecl[1], L. Bacer-Kermavner[1], J. Mivsek[1], P. Petric[1], N. Jancar[1], E. Vrtacnik-Bokal[1]

[1]Univerzitetni Klinicni Center Ljubljana, Department of Obstetrics and Gynaecology, Ljubljana, Slovenia

Study question: What is the proportion of the unused supernumerary cryopreserved embryos and couples, who don't use their cryopreserved embryos after IVF/ICSI treatment?
Summary answer: After five years of cryostorage, one third of couples still have their supernumerary embryos cryopreserved, and also one third of all embryos stay cryopreserved.
What is known already: With improvements in IVF treatments the number of cryopreserved supernumerary embryos also increased, and consequently more and more embryos are left in liquid nitrogen or discarded after certain time. The practice usually depends on legislation, if there is one; otherwise the IVF centres decide by themselves what to do. In our country, the national law stipulates that embryos can be frozen for five years, and after that discarded, although there is possibility to extend the cryopreservation time for additional five years. Despite well-defined conditions in our law, about how long to cryopreserve embryos, this still opens some ethical concerns.
Study design, size, duration: In January 2020, we retrospectively collected the data of all fresh cycles which led to embryo cryopreservation, in couples treated in our centre from January 2014 to December 2018, and analysed how many embryos are still frozen.
Participants/materials, setting, methods: We checked what the dynamic of thawing embryos was, in terms of the proportion of thawed embryos depending on cryostorage time. We also analysed what is the proportion of couples who still have frozen embryos even after five years of cryostorage, which is a legislative limit (although cryostorage is usually prolonged for additional five years and after that time the embryos are discarded).
Main results and the role of chance: The data show that after the first year of cryostorage (embryo cryopreservation in 2018) 42% of embryos were thawed, after two years (cryopreservation in 2017) 50%, after three years (cryopreservation in 2016) 54%, after four years (cryopreservation in 2015) 64%, and after five years (cryopreservation in 2014) 59%. The proportion of couples who used all their cryopreserved embryos after first year of cryostorage (embryo cryopreservation in 2018) was 42%, after two years (cryopreservation in 2017) 52%, after three years (cryopreservation in 2016) 53%, after four years (cryopreservation in 2015) 51% and after five years (cryopreservation in 2014) 59%.

We additionally tried to analyze, why so many embryos by so many couples (41%) are left frozen even after 5 years of cryostorage (cryopreservation in 2014). Our analysis indicates that one of the reasons is probably that couples conceived after fresh or frozen embryo transfer and just left other embryos frozen (28%). There is also a proportion of couples who didn't conceive through IVF, but still left their embryos frozen, sometimes even without single frozen/thawed embryo transfer (13%). They probably conceived spontaneously, or there was some other unknown reason.
Limitations, reasons for caution: The limitation of the study is retrospective design and that the proportions of thawed embryos and couples using these embryos were not followed each year separately, but for all years together.
Wider implications of the findings: The couples could be encouraged to use each and every one of their cryopreserved embryos, or to donate their embryos to other couples in the countries where this is allowed.
Trial registration number: not applicable

P-736 Import and Export of Gametes and Embryos in Italy: retrospective data realignment and implementation of a new data collection platform

G. Capelli[1], S. Bolli[2], L. Santilli[3], F. Bariani[3], V. Vigiliano[2], R. De Luca[2], L. Lombardini[3], M. Cardillo[3], G. Scaravelli[2]

[1]Italian National Health Institute - ISS / University of Cassino e Lazio Meridionale, Observatory for Medically Assisted Reproduction / Department of Human Sciences- Society and Health, Rome / Cassino FR, Italy ;
[2]Italian National Health Institute - ISS, Italian National ART Registry, Rome, Italy ;
[3]Italian National Health Institute - ISS, Italian National Transplant Centre - CNT, Rome, Italy

Study question: How many gametes and embryos travel among countries, due to reasons ranging from gamete donation supply levels to barriers in accessing specific healthcare procedures?
Summary answer: Reliable data on cross-border circulation of gametes and embryos are extremely important, also to evaluate lack of national donations.
What is known already: While some studies point out the importance of issues linked to reproductive healthcare tourism or trans-border circulation of gametes and embryos, few data are available on the matter. In Italy, since 2012, the law commits ART centers to notify any activity of Import and Export of gametes and embryos to both the Italian ART Registry in the Italian National Health Institute (ISS), which is in charge of collecting data on ART activities in Italy and the Italian National Transplant Centre (CNT), which is in charge of ensuring safety and quality of cells and tissues in Italy.
Study design, size, duration: After 2014, ART treatments with donated gametes were allowed by law in Italy, and gamete Import/Export activities started to rise. In 2017, the Italian ART Registry, the CNT, and the ART Observatory in ISS, started a retrospective cross-validation of Import/Export databases separately collected in 2016 and 2017 and planned and implemented a new common web-based system, accessible by the ~350 Italian ART centers, which came into force on 01/01/2018, to allow prospective data collection.
Participants/materials, setting, methods: Two different databases, imputed both in 2016 and 2017 on Excel files by ART Registry and CNT separately, were checked, homogenized and imported and analyzed by Stata 15. Meanwhile, the working group defined a common dataset and implemented a web-based interface for the prospective data collection, in which Single ART Establishments started to impute data prospectively on a day-by-day basis since 01/01/2018.
Main results and the role of chance: In 2016 and 2017, a total of 3040+3063 straws/vials of semen, 6239+6731 straws/vials of oocytes and 2865+2632 embryos were imported to Italian ART Centers from foreign gamete Banks and ART Centers, and a total of 3581+2937 straws/vials of semen, 16+33 straws/vials of oocytes and 55+57 embryos were exported from Italian ART Centers to foreign ART Centers. Tables, heatmaps and other data visualization tools were used to show the straws/vials imported and exported, stratified by Region for the Italian ART Centers, and by Country (both EU and extra-EU) for the International ART Centers. Many Italian ART centers work mainly importing semen and/or oocytes, while some specific centers show significant volumes of semen export followed by an import of frozen embryos. All these data were included in an Appendix of the 2018 and 2019 Annual Reports of the Italian Ministry of Health on ART activities.

Data collected from January 2018, thanks to a robust web-based data interface, will allow also to identify even better, through the analysis of SEC codes, the cross-border path followed by gametes and embryos. 2018 data will be presented in the 2020 Annual Report of the Italian Ministry of Health on ART activities, due for 30/06/2020.
Limitations, reasons for caution: In 2016 and 2017 data, some underreporting and misclassification may have occurred, since some ART Centers were regularly notifying import/export either to the Italian ART Registry or the CNT, and some misclassification of International Centers operating in the same country and belonging to the same Company may have occurred.
Wider implications of the findings: "Mapping" of the gamete and embryo cross-border exchange paths may help to identify and study international networks of co-operating ART Centers. Moreover, the need to notify import and export activities through the new web-based interface is increasing the awareness about the EU Compendium and the SEC codes.
Trial registration number: not applicable

P-737 Assessing women's preferences in a novel intrauterine device designed to monitor the womb environment in real time: A discrete choice experiment

K.Y.B. Ng[1,2], R. Evans[2], S. Wellstead[2], A. Burtt[2], E. Mentzakis[3], Y. Cheong[1,2]

[1]University of Southampton, School of Human Health and Development, Southampton, United Kingdom ;
[2]University Hospitals Southampton NHS Foundation Trust, Department of Obstetrics and Gynaecology, Southampton, United Kingdom ;
[3]University of Southampton, Department of Economics, Southampton, United Kingdom

Study question: What factors influence a woman's decision to use an intrauterine device designed to monitor the uterine environment?
Summary answer: Willingness to use a novel intrauterine device to monitor the in-vivo uterine environment is mostly influenced by its ability to guide treatment of reproductive failures.
What is known already: There is increasing evidence suggesting that the intrauterine environment is essential for successful implantation and placentation. The University of Southampton, in conjunction with an industry partner, are developing an intrauterine device which has the potential to monitor the temperature, pH and dissolved oxygen in real time in the uterus, with the aim of supporting those with reproductive failures. The device is similar in shape and size to a contraceptive coil, and has an accompanying wearable garment with an information receiver to collect data. This study aims to explore factors that are of importance to potential users of the intrauterine device.
Study design, size, duration: A discrete choice experiment (DCE) was conducted; DCE is a preference elicitation method whereby individuals reveal their preference over selected attributes of a product in a series of pair-wise choices of hypothetical alternative products. Choice sets consisted of two hypothetical intrauterine devices described via four attributes: length of use, information obtained and its use in guiding treatment, risk of complications and discreteness of the information receiver. Ethical approvals were obtained (ERGO 49115, REC 19/IEC08/0036).
Participants/materials, setting, methods: 220 women of reproductive age (18-50 years), who were wishing to achieve a pregnancy now or sometime in the future were recruited at Princess Anne Hospital in Southampton. A fractional factorial design was used to estimate effects of the four attributes, using 18 pair wise choice sets that identified all main effects and selected two-way interactions, blocked into two blocks of 9 choice sets each. Conditional and mixed logit models were used to analyse responses.
Main results and the role of chance: Analysis was performed through conditional and mixed logit models with similar results. Overall, women prefer a device which stays in the uterus for a shortest length of time, provides information that guides treatment in all cases, has the lowest risk of complications, and has an information receiver which is completely discrete. All such preferences were statistically significant ($p<0.05$). Examining relative importance attached to the attributes, women placed most importance on the information obtained from the device and its ability to guide treatment. Specifically, respondents were over 2 times more likely to choose a device if the information obtained guided treatment in all cases, compared to when information obtained guided treatment in majority of cases. Obtaining information that guided treatment in all cases had an equal importance to a drop in risk of complications by 15%. Further, the impact of a 1% increase in the risk of complications was comparable to a 7 day increase in the length of use. Finally, moving from a moderately discrete to an indiscrete information receiver reduced the device's attractiveness by a third.
Limitations, reasons for caution: Hypothetical bias may lower external validity. Although we obtained data on the 'stated' preferences within the hypothetical choice sets, we were not able to capture the 'actual' preferences of the women from this study.
Wider implications of the findings: Before the introduction of a novel fertility assessment tool, it is important to gain knowledge of the preferences of potential users. The usefulness of the device in obtaining information that will guide treatment of reproductive failures is of paramount importance to users, and appropriate counselling will support informed decision making.
Trial registration number: N/A

P-738 Age-related natural fertility outcomes in women over 35: an individual participant data meta-analysis

B.W. Mol[1], S.J. Chua[2], N. Danhof[3], M. Mochtar[3], M. Van Wely m.vanwely@amsterdamumc.nl[3], D. Mclernon d.mclernon@abdn.ac.uk[4], I. Custers i.m.custers@amc.uva.nl[3], E. Lee[5], K. Dreyer[6], D. Cahill[7], W. Gillett[8], A. Righarts[9], A. Strandell annika.strandell@medfak.gu.se[10], R. Van Eekelen[11], T. Rantsi tiina.rantsi@hus.fi[12]

[1]Monash Medical Centre- Monash Health and Monash University, Obstetrics and Gynaecology, Melbourne, Australia ;
[2]University of Adelaide- Adelaide- South Australia- Australia 5000, Obstetrics & Gynaecology, Adelaide, Australia ;
[3]Academic Medical Centre- University of Amsterdam, Department of Obstetrics & Gynaecology, Amsterdam, The Netherlands ;
[4]Institute of Applied Health Sciences- University of Aberdeen, Medical Statistics Team, Aberdeen, United Kingdom ;
[5]Western Ultrasound for Women, Ultrasound, West Leederville, Australia ;
[6]Vrije Universiteit Amsterdam, Department of Reproductive Medicine, Amsterdam, The Netherlands ;
[7]St Michael's Hospital, Academic Unit of Obstetrics & Gynaecology, Bristol, United Kingdom ;
[8]Dunedin School of Medicine- University of Otago, Women's and Children's Health, Dunedin, New Zealand ;
[9]Dunedin School of Medicine- The University of Otago, Women's and Children's Health, Dunedin, New Zealand ;
[10]Institute of Clinical Sciences- Sahlgrenska Academy- University of Gothenburg, Department of Obstetrics and Gynecology, Goteborg, Sweden ;
[11]Academic Medical Center- University of Amsterdam, Department of Obstetrics and Gynaecology, Amsterdam, The Netherlands ;
[12]University of Helsinki and Helsinki University Hospital, Department of Obstetrics and Gynecology, Helsinki, Finland

Study question: What is the rate of natural conception leading to livebirth over 6–12 months for infertile women of age ≥ 35 years?
Summary answer: Natural conception rates were still clinically relevant in women aged 35 and above and were significantly higher in women with unexplained infertility.
What is known already: In recent years, increasing numbers of women have attempted to conceive at a later age, resulting in a commensurate increase in the need for assisted reproductive treatment (ART). However, there is a lack of data on natural fertility outcomes (i.e. no interventions) in women with increasing age.
Study design, size, duration: Systematic review with individual patient data meta-analysis. PubMed, Medline, Embase, the Cochrane Library, clinicaltrials.gov were searched until 1st July 2018 including search terms "fertility service", "waiting list", "treatment-independent", "spontaneous conception". Language restrictions were not imposed.
Participants/materials, setting, methods: Inclusion criteria were studies reporting on infertile couples with female partner of age ≥ 35 who attended fertility services, underwent fertility work-up and were exposed to a period of expectant management. For studies that met inclusion criteria, study authors were contacted to provide individual participant data. Time to pregnancy or live birth and the effect of increasing age on fertility outcomes after adjustment for other prognostic factors were analysed.
Main results and the role of chance: We included 10 studies (7 cohort studies and 3 randomised controlled trials) (n=4415 women of at least age 35), with the observed composite primary outcome of ongoing pregnancy or live birth occurring in 436 women (9.9%) over a median follow-up of 5 months (P25-75: 2.5-8.5). Studies were of moderate to high quality. The probability of natural conception significantly decreased with any diagnosis of infertility, when compared with unexplained infertility. We found non-linear effects of female age and duration of infertility on natural conception and tabulated the predicted probabilities for unexplained infertile women of ages 35 to 42 with either primary or secondary infertility and with a duration of infertility from 1 to 6 years. For a 35-year-old woman with 2 years of primary unexplained infertility, the predicted probability of natural conception leading to live birth was 0.15 (95%CI 0.10–0.19) after 6 months and 0.23 (95%CI 0.17–0.29) after 12 months. For a 42-year-old woman, this decreased to 0.07 (95%CI 0.04–0.11) after 6 months and 0.12 (95%CI 0.07–0.17) after 12 months.
Limitations, reasons for caution: There were different study designs, recruitment strategies in different centres, protocols and countries and different methods of assessment of infertility utilised. Data was limited for women above the age of 40.
Wider implications of the findings: Women attending fertility services should be encouraged to pursue natural conception while waiting for treatment to commence and after treatment if it is unsuccessful. Our results may aid in counseling women, and, in particular, those with unexplained infertility.
Trial registration number: N/A

P-739 Dietary glycemic load and unexpected poor response to ovarian hyper-stimulation: a prospective cross-sectional study

S.A. Noli[1], E. Ricci[2], S. Ferrari[3], M. Reschini[3], F. Parazzini[4], E. Somigliana[3]

[1]Università degli Studi di Milano- Milan- Italy, Department of Clinical Sciences and Community Health, Milan, Italy ;
[2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Department of Woman- Newborn and Child, Milan, Italy ;
[3]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Fertility Unit, Milan, Italy ;
[4]Università degli Studi di Milano, Department of Clinical Sciences and Community Health, Milan, Italy

Study question: Is there any association between dietary glycemic load and unexpected poor ovarian response to hyper-stimulation?
Summary answer: Elevated dietary glycemic load is associated with unexpected poor ovarian response.
What is known already: In recent years, increasing interest was turned to the relation between nutrition and fertility. Several studies suggested that diet in general and glycemic load in particular can affect oocytes quality, embryo development and time to pregnancy, after both natural and assisted reproduction. However, evidence is not univocal. Of relevance here is that pregnancy is the result of several factors, of whom most may not be influenced by diet. Investigations specifically focusing on particular aspects of reproduction are therefore needed. In this study, we tested the possibility that diet may cause poor response in women with normal biomarkers of ovarian reserve.
Study design, size, duration: Women eligible for in IVF at an Academic Fertility Center were invited to participate to a prospective cross-sectional study on the role of diet on IVF outcomes. Main inclusion criteria were: age between 18 and 39 years, BMI >18 and <25 Kg/m^2, preserved ovarian reserve (anti-muellerian hormone (AMH) between 2 and 5 ng/mL and/or total Antral Follicle Count (AFC) between 10 and 22 and starting dose of gonadotropins between 150-225 IU/day.
Participants/materials, setting, methods: Information on diet was obtained using a validated food frequency questionnaire (FFQ). *Unexpected poor ovarian response* was defined as the retrieval of ≤3 suitable oocytes in women expected to be normal responders based on AFC and AMH. A logistic regression model was used to adjust for confounders.
Main results and the role of chance: Out of 303 women undergoing IVF and enrolled in the study, 48 (16%) showed poor response to ovarian hyper-stimulation. Main baseline characteristics of the study groups did not significantly differ. The number of poor responsive subjects increased with glycemic load with borderline significance, both when analyzed in tertiles (p=0.054) and as continuous variable (p=0.08). However, when adjusting for age, BMI, education, smoking, occupational and leisure physical activity, endometriosis, daily calories intake, fibers intake, caffeine intake and alcohol intake, the association became statistically significant (p for trend, p=0.02). Specifically, when comparing the third to the first tertile (reference) of glycemic load, the adjusted OR was 3.91 (95% CI: 1.11-13.83).
Limitations, reasons for caution: Three main limitations should be considered: 1) findings should be referred only to women of infertile couples; 2) although we used a previously validated FFQ, information regarding dietary habits were self-reported; 3) the design of study does not allow to conclude for a causal relation.
Wider implications of the findings: If confirmed, a detrimental role of diet on ovarian response and in particular on the proportion of women with unexpected poor response may open to new therapeutic approaches. Future interventional studies should clarify whether diet modification may restore normal response to ovarian hyper-stimulation.
Trial registration number: not applicable

P-740 Overestimated? Prevalence of thrombophilia and antiphospholipid syndrome in patients with recurrent miscarriage and healthy controls

K. Vomstein[1], A. Herzog[1], P. Voss[2], K. Feil[1], T. Goeggl[2], T. Strowitzki[2], B. Toth[1], R.J. Kuon[2]

[1]Medical University Innsbruck, Department of Gynecological Endocrinology and Reproductive Medicine, Innsbruck, Austria ;
[2]Ruprecht-Karls University Heidelberg, Department of Gynecological Endocrinology and Fertility Disorders, Heidelberg, Germany

Study question: Does the prevalence of inherited and acquired thrombophilia differ between patients with recurrent miscarriage (RM) and healthy controls?
Summary answer: There is no difference in the prevalence, neither in inherited nor in acquired thrombophilia between RM patients and healthy controls.
What is known already: Although frequently performed, routine screening for inherited thrombophilia in patients with recurrent miscarriage is not recommended in clinical guidelines, whereas screening for Anti-phospholipid Antibody Syndrome (APS) is highly recommended. Further, studies on the frequency of thrombophilia in RM patients showed contradictory results. Inherited and acquired thrombophilia are associated with severe pregnancy complications like preeclampsia, fetal growth restriction and stillbirth as well as increased maternal risks of venous thromboembolism with serious long-term consequences. There is an ongoing debate on the impact of these thrombophilia on RM with the presumed mechanism of a thrombosis within the uteroplacental circulation.
Study design, size, duration: At two university-based Departments between 01/2011 and 07/2019, n=820 patients with RM (defined as ≥ 3 consecutive miscarriages) were included within a multi center case control study. The control group (n=141) consisted of young healthy women who underwent a routine screening for inherited and acquired thrombophilia. Diagnostics were performed in non-pregnant RM patients and controls.
Participants/materials, setting, methods: In non-pregnant RM patients and controls, obstetric and medical histories were obtained (including age, body mass index (BMI), gravidity, parity, number of miscarriages). The prevalence of inherited and acquired thrombophilia including deficiency of protein C/S and antithrombin (AT), elevation of factor VIII activity, APC resistance/ mutation in the factor V Leiden (FVL, G1691A) gene, mutation in the prothrombin (G20210A) gene and antiphospholipid antibodies were assessed and compared between RM patients and controls.
Main results and the role of chance: RM patients were significantly older than controls (mean age±SD, 34.24±4.59 vs 5.56±1.92, p<0.0001). The BMI in RM patients was higher than in controls (24.30±4.45 vs. 21.54±4.4, p<0.001). RM patients had a history of 3 (3/14) miscarriages (median (min/max)). There were no differences concerning the number of previous miscarriages between RM patients between the two Departments. No differences in the prevalence of protein C/S deficiency, FVL or prothrombin gene mutation were observed in RM patients and controls. However, homozygous mutations were identified only in the RM group (RM vs. controls in %: FVL heterozygous/homozygous: 7.4/0.26 vs 7.1/0.0; prothrombin heterozygous/homozygous: 3.69/0.53 vs 0.71/0.0; deficiency of protein C: 0.39 vs 0.00, protein S: 2.77 vs 3.55, AT: 0.76 vs 1.5; all p>0.05). The frequency of antiphospholipid antibodies did not differ significantly in both groups (RM vs. controls in %: anti-cardiolipin antibodies 3.66 vs. 0.0; anti-β2-glycoprotein antibodies 2.5 vs 0.7; lupus anticoagulant 0.78 vs 0.7; all p>0.05). Eleven patients in the RM group, but none of controls, fulfilled the Sydney criteria and were diagnosed with an APLS (prevalence of APLS 1.44% vs 0%, p=0.38). An elevation of factor VIII was significantly more prevalent in controls (RM vs controls in %: 5.8 vs 11.0, p=0.038).
Limitations, reasons for caution: This study is not designed to explain any possible causality between thrombophilia and RM. As our controls were of younger age and nulliparous, neither a possible influence of a future pregnancy, nor a possible development of an acquired thrombophilia or future recurrent miscarriage (prevalence of 1-3%) can be excluded.
Wider implications of the findings: The prevalence of inherited and acquired thrombophilia in RM patients has been overestimated in previous studies. While earlier studies identified an association of some inherited thrombophilia with RM, our data do not support these findings. Still, we recommend screening for APLS as it has deleterious effects on implantation and pregnancy.
Trial registration number: not applicable

P-741 The impact of smoking in 2,000 recurrent pregnancy loss patients

K. Hviid[1], A.M. Kolte[2], M.E. Issaoui[2], M.C. Krog[2], H.S. Nielsen[3]

[1]Fertility Clinic 4071- Rigshospitalet- Copenhagen University Hospital- Blegdamsvej 9- DK-2100 Copenhagen Ø- Denmark. Dept. of Obstetrics and Gynaecology- Hvidovre Hospital- Kettegaard Alle 30- DK-2650 Hvidovre- Denmark, Dept. of Obstetrics and Gynaeco, ;
[2]Fertility Clinic 4071- Rigshospitalet- Copenhagen University Hospital- Blegdamsvej 9- DK-2100 Copenhagen Ø- Denmark, Fertility Clinic, Copenhagen, Denmark ;
[3]Dept. of Obstetrics and Gynaecology- Hvidovre Hospital- Kettegaard Alle 30- DK-2650 Hvidovre- Denmark. Dept. of Clinical Medicine- University of Copenhagen, Dept. of Obstetrics and Gynaecology-, Hvidovre, Denmark

Study question: Are pregnancy history and the first outcome after referral different in smoking recurrent pregnancy loss (RPL) patients versus never-smokers?
Summary answer: Smoking RPL patients were referred younger, with more pregnancy losses and stillbirths compared to never-smokers but had no significant difference in live birth after referral.
What is known already: Smoking during pregnancy is associated with negative reproductive effects including low birthweight, placenta abruption, preterm delivery, shortened gestation, stillbirth, and sudden infant death syndrome. A recent meta-analysis examined the effect of maternal smoking on pregnancy outcomes after assisted reproductive technology and reported a decreased rate of both live birth and of clinical pregnancy respectively, and a higher pregnancy loss rate per pregnancy in the smoking group. The impact of smoking in RPL patients has not yet been elucidated.
Study design, size, duration: A cohort study including women referred to the Recurrent Pregnancy Loss Unit, the Fertility Clinic, Rigshospitalet, Copenhagen University Hospital, Denmark, between 01 January 2000 and 31 December 2017, and under the age of 42 years at referral. The cohort consists of 2,138 women with RPL. Follow-up of the first pregnancy after referral ended by December 2018.
Participants/materials, setting, methods: Pregnancy history and smoking behaviour is systematically collected at the first consultation in the RPL unit and with consent saved in a dedicated database. Patients were divided into 'smokers at time of referral' or 'never-smokers'. Former or occasional smokers were excluded. The outcome of the first pregnancy after referral was categorised as live birth versus pregnancy loss. Where appropriate the following statistical analyses were used: independent samples t-test, Pearson Chi-Square, Mann-Whitney, and multiple logistic regression.
Main results and the role of chance: Smokers at time of referral were on average 2.12 years younger than never-smokers at referral for RPL ($P < 0.0005$) and had significantly more losses prior to referral than never-smokers ($P < 0.002$). Among women with secondary RPL, smokers at time of referral's first-born children had a significantly lower birth weight and had experienced more stillbirths prior to referral ($P = 0.027$ and $P = 0.01$, respectively).

Among the 1,360 women with a pregnancy after referral in the RPL Unit 58% of smokers at time of referral had a live birth compared with 62% of never-smokers ($P = 0.289$). In an adjusted analysis no significant association was found between smoking status and chance of a live birth (OR 1.22, 95% CI: 0.88-1.68). In the first pregnancy after referral, stillbirth was significantly more common among smokers than never-smokers ($P = 0.004$). There were no statistically significant differences in birth weight or gestational age in the first pregnancy after referral between smokers at time of referral and never-smokers.
Limitations, reasons for caution: A weakness in our study is that we very strongly recommend smoking cessation at the first consultation, and our next encounter with the patient is when they report the next pregnancy. Patients who quit smoking before the first pregnancy after referral are thus erroneously categorized as active smokers.
Wider implications of the findings: Smoking RPL women are referred younger, with more pregnancy losses and stillbirths. As we lack data on smoking cessation after referral, we are uncertain if smoking reduces chances of live birth, however, the first pregnancy after referral smokers had more stillbirths. Smoking seems to be a risk factor for RPL.
Trial registration number: Not applicable

P-742 Risk factors that increase twin pregnancies following single embryo transfer with a blastocyst in relations to ART

T. Eliasen[1], A. Gabrielsen[1], B. Bay[1], U. Knudsen[1]

[1]Horsens, Fertility Clinic, Aarhus, Denmark

Study question: Which risk factors increase the risk of twin pregnancies following fertility treatment with single embryo transfer (SET) at the blastocyst stage?
Summary answer: Lower maternal age showed to be significant associated to increased risk of having twins in women following SET.
What is known already: The incidence of monozygotic (MZ) twins after fertility treatment has been reported to be higher than that following natural conception. Due to the increased maternal and fetal risk in twin pregnancies, reducing the risk of twin pregnancy, especially monochorionic, is important. Factors that have been hypothesized to increase the risk of MZ twins include fertilization method in ART, maternal age, oocyte age, embryo morphology, and culture media but studies have shown equivocal results.
Study design, size, duration: This study is a retrospective cohort study. Women who received fertility treatment from 2010-2019 at two fertility clinics in central Denmark were included. A total of 4,214 women had received 6,502 SET at the blastocyst stage with 2,196 (33.8%) subsequent pregnancies and of those 84 (3.8%) had twin pregnancies.
Participants/materials, setting, methods: Information on the women was collected from treatment start and during the pregnancy. The information included maternal age, indication for treatment, stimulation protocol, fertilisation method, BMI, blastocyst score prior to transfer, and ultrasonic findings at gestational week 8. Ultrasonic findings were confirmed by reading each patient's medical journal.
Main results and the role of chance: Twin pregnancies was identified in 84 women, and of these 43 were ultrasonically confirmed at gestational week 8 to be MC, 13 were dichorionic (DC) and 28 were of uncertain of the choriosity. This resulted in a MC twinning rate of 1.96% and a rate of 0.59% of having a DC twin. Lower maternal age was associated with an increased risk of having twins when comparing both DC and MC twins to singletons ($p=0.04$) but not when comparing only MC twins to singleton ($p=0.10$). There was no difference in the use of ICSI vs IVF. Furthermore, there was no observed difference in indication of treatment and twin rates. Finally, no difference in maternal or paternal BMI was observed.
Limitations, reasons for caution: Only ultrasonically confirmed MC pregnancies were included, which allows for a risk of underestimation, as many of the pregnancies with uncertain choriosity could be MC. Furthermore, the limited number of twin pregnancies makes unveiling significant risk factors difficult.
Wider implications of the findings: Twin rates following ART remains higher than that of natural conception. This report on twins following SET of a blastocyst found lower maternal age to be a risk factor. Assisted reproductive therapy procedure, BMI, or maternal indication was not found to be risk factors.
Trial registration number: STPS 3-3013-3234/1

P-743 Are clinical pregnancy and live birth rates comparable to evaluate the effectiveness of Assisted Reproductive Technologies?

F. Lorenzo[1], M.P. Zappacosta Villarroel[2], M.A. Armani[2], J.T. Garcia[2], S. Bouzas[3], A. Valcarcel[3]

[1]IFER, Reproduction, Buenes Aires, Argentina ;
[2]IFER, Reproduction, Ciudad Autonoma de Buenos Aires, Argentina ;
[3]IFER, Biology, Ciudad Autonoma de Buenos Aires, Argentina

Study question: Measuring outcomes: Are clinical pregnancy rate and live birth rate comparable to evaluate the effectiveness of Assisted Reproductive Technologies?
Summary answer: Conclusions on the results of a treatment based on clinical pregnancy or live birth rates as primary endpoints are comparable.
What is known already: The endpoint of an Assisted Reproductive Technology treatment is the live birth of a healthy baby. However, sometimes follow up until live birth is an additional challenge for the investigator, because couples usually return to a local obstetrician for medical care. Moreover, many studies report pregnancy rates instead of live birth rates, but this would underestimate the number of live born babies.
Study design, size, duration: This is an observational restrospective single-centre study that included 2473 women under 40 years old who underwent In Vitro Fertilization fresh embryo transfers, from January 2014 until December 2018. Statistical analysis was performed using the Chi Square test.
Participants/materials, setting, methods: Primary outcome was to compare live birth with clinical pregnancy rate. Live birth rate was defined as the birth of a living baby after embryo transfer. Clinical pregnancy rate was defined by ultrasonographic documentation of at least one fetus with a heart beat at 6-7 weeks of gestation. Secondary outcomes where miscarriage rate (intrauterine pregnancy loss before 20 weeks of gestation), implantation rate (number of gestational sacs observed at vaginal ultrasound/number of transferred embryos).

Main results and the role of chance: This study included 2473 women who underwent In vitro Fertilization fresh embryo transfers, regardless of the number of oocytes collected. Average age was 37,6 ± 2,1 years old. A total of 11351 oocytes were collected (mean 4,6 ± 2,5 SD). An average of 2.1 ± 0,4 embryos were transferred. Clinical pregnancy rate was 18% (452/2473) and live birth rate was 17% (411/2473) p: 0,13. Implantation rate was12% (570/4950). Miscarriage rate was 9 % (41/452).Conclusions on the effectiveness of a treatment based on either clinical pregnancy rate o live birth rate as endpoints are comparable.
Limitations, reasons for caution: This is a restrospective single - centre study.
Wider implications of the findings: As a large number of trials in reproductive medicine report pregnancy rates and data regarding to de future new born may be not be available, clinical pregnancy rate could be an optimal parameter to report assisted reproduction technologies results.
Trial registration number: not applicable

P-744 Setting a tariff for in-vitro fertilisation and intracytoplasmatic sperm injection (IVF/ICSI) in Scotland: a cost analysis

R. Venson[1], A. Maheshwari[2], S.M. Nelson[3], S. Corcoran[4], K.A. Boyd[1]

[1]University of Glasgow, Health Economics and Health Technology Assessment, Glasgow, United Kingdom ;
[2]National Health Service Grampian, Aberdeen Maternity Hospital, Aberdeen, United Kingdom ;
[3]University of Glasgow, School of Medicine, Glasgow, United Kingdom ;
[4]Scottish Government, Maternal & Infant Health, Glasgow, United Kingdom

Study question: What is the full economic cost of IVF/ICSI fresh and frozen cycles accounting for equipment, staff, medication, consumables, and overheads in Scotland?
Summary answer: The average full economic cost of fresh cycles was £3,334. Fresh IVF and ICSI averaged £3,185 and £3,459, respectively. Frozen cycles were cheaper, averaging £872.
What is known already: There is a need for a standardisation of price for IVF/ICSI. There is a lack of published economic studies in this field. IVF/ICSI is a very complex process that includes many different scenarios, which makes the costing process multifaceted and hinders the interpretation of the results. According to benchmarking performed for prices in NHS-England, IVF and ICSI cycles (including a fresh and a frozen cycle) cost £3,100-£3,500 and £3,500-£4,000, respectively; each subsequent frozen cycles should cost around £1,000, however, the published document was not transparent on the methods adopted to estimate these costs, or what costs were included.
Study design, size, duration: Bottom-up costs were calculated using cycle-level resource data from all Centres in Scotland during the period of 01/Jan/2015 to 31/Jul/2018. Whenever micro-costing was not possible due to the nature of the data available, aggregated macro-level data analysis was adopted.
Participants/materials, setting, methods: Anonymised cycle-level data was obtained from the four Scottish Centres that provide NHS-funded IVF/ICSI (cycles between 01/Jan/15-31/Jul/18). Costs were calculated in 2018 UK sterling prices. Resource use were assigned to individual cycles based on cycle-level data or expert-informed assumptions; when this was not available, average aggregate costs were assigned to each cycle. Cycles cancelled at early stages were also included in the analysis. Sensitivity analysis varying the ratio frozen embryo transfer:fresh cycles was performed.
Main results and the role of chance: A total of 9,442 NHS-funded cycles were included in the analysis (Ab: 1,888; Dun: 1,566; Ed: 2,224; Gla: 3,766). The average age was 34.0±4.3 years, with most of the fresh cycles (82%) being for patients below 40 years of age. Approximately 40% were IVF (14% for Gla and 50-70% for the other Centres) with the remaining being ICSI. Fresh cycles comprised 70% (62%-74%) of all cycles, whilst the rest were frozen cycles. The average cost of a fresh cycle was £3,334 (range £1,742-£4,548); with fresh IVF costing £3,185 (range £1,742-£4,000) and a fresh ICSI costing £3,459 (range £1,742-£4,548). Frozen cycles were cheaper, averaging £872 (range £207-£1,032). Cheaper cycles within the presented ranges were cycles cancelled at early stages due to various reasons. On average, 0.46 frozen ET happened for every fresh cycle. This proportion was used to estimate the average cost of a full cycle per patient, which was £3,715. The sensitivity analysis showed that, if all Centres have one frozen embryo transfer for every fresh cycle, the full cycle (1 fresh + 1 frozen) would cost, on average, £4,236.
Limitations, reasons for caution: The main challenge was finding a balance between the costing and incorporating the Centre available data and specific practices. This study was based on typical IVF/ICSI processes, and did not include specialised treatment or cost of adverse events. Assumptions were made throughout the study; these are inherent to costing studies.
Wider implications of the findings: The results presented in this study should serve as a guide to NHS-Scotland to set a tariff in the country. However, decisions made based on this study should be careful, bearing in mind the dynamicity of ART, due to constant changes in procedures and policies and new technologies being adopted.
Trial registration number: not applicable

P-745 The frozen egg's journey, does it change their efficiency?

G. Scaravelli[1], R. De Luca[1], R. Spoletini[1], V. Vigiliano[1], L. Speziale[1], S. Bolli[1], A. Bertini[1], S. Fiaccavento[1]

[1]Istituto Superiore di Sanita, National Centre for Diseases Prevention and Health Promotion, Rome, Italy

Study question: Do the female gametes transportation and cryopreservation process influence the future success or efficiency in a donor ART treatment?
Summary answer: Fresh or frozen egg donor cycles report the same results when combined respectively with FER or with fresh embryo transfer.
What is known already: The debate is still open whether fresh donor eggs are more efficient than frozen ones in an ART treatment. In Italy, since 2012 import and export of gametes and embryos is allowed as ART donor cycles from 2014. Nevertheless, due to the lack of information campaign, and to the prohibition of compensation to donors, we have no donors. Then, all the gametes utilized in Italy in donor cycles are imported from abroad and the majority of them are frozen egg cycles. Some Italian Centers, to utilize fresh eggs, send the semen abroad and import back the embryo for transfer.
Study design, size, duration: The Italian Assisted Reproduction Techniques Register(IARTR) analyzed retrospectively summary data on 11,144 oocytes donation cycles performed from 2017 to 2018 on 9,675 patients. 106 ART clinics sent data on gametes donation cycles to the IARTR and participated in the study with 97 centers (91.5%) performing at least one oocytes donation cycle. A comparison was made on the efficiency of fresh and frozen egg donor cycles also considering fresh or frozen embryo condition at transfer.
Participants/materials, setting, methods: All ART centers which have performed at least one cycle with gametes donation, that have sent data during the study period were included. Parameters regarding number of patients, number of cycles, fresh or cryopreserved ones, treatment indications, age classes, pregnancies, deliveries and live births rates were statistically analyzed using SPSS statistic 25.0. Differences between groups were compared with chi-square test and p-value <0.05 was considered to be statistically significant.
Main results and the role of chance: 97 centers with at least one donor egg cycle participated to the data collection, only 18 were public or private covered by NHS (18.6%). 9675 patients underwent 11144 cycles with egg donation, 8108 cycles (72.8%) were frozen donor eggs cycles and 3036 (27.2%) were fresh. Main indications for treatment with oocytes donation were: maternal advanced reproductive age (36.6%) and reduced ovarian reserve (36.2%). Almost all (97.9%) the oocytes used come from abroad.10414 transfer were performed (93.4% of initiated cycles) with mean age of women at transfer that was 41.6 years. The 98.4% of transfers in fresh donor eggs cycles were frozen embryo replacement (FER), while only the 23.5% in frozen donor eggs cycles. Pregnancy rates per transfer were similar if we compared treatments where only one cryopreservation take place: 35.6% with fresh eggs + FER and 36.4% with frozen eggs + fresh embryos. Cycles utilizing frozen eggs + FER show significantly lower rates 28.1% (p<0.01). Fresh eggs + FER reported significantly higher rates than the other for delivery rates (25.5%, 23.4% and 16.4% respectively) and for live birth rates (25.3%, 23.3% and 16.4% respectively). In these 2 years 2681 children were born alive with egg donation.
Limitations, reasons for caution: this is a retrospective study made with a summary data collection.

Wider implications of the findings: Our results showing slight differences in the outcomes between the group of egg donor cycles fresh or frozen, when only one cryopreservation process takes place i.e. fresh eggs plus FER and frozen egg plus fresh embryo transfer, could suggest new strategies in donor art cycles.
Trial registration number: not applicable

P-746 Probability of a live birth after fertility treatments in female cancer survivors - A Finnish population-based registry study

J. Melin[1], L. Madanat-Harjuoja[2], E. Hirvonen[3], N. Malila[4], J. Pitkäniemi[3], M. Gissler[5], A. Tiitinen[6]

[1]University of Helsinki- Helsinki University Hospital, Department of Obstetrics and Gynecology, Vantaa, Finland ;
[2]Harvard Medical School, Dana Farber Cancer Institute/Boston Children's Cancer and Blood Disorders Center- Boston, Boston- MA, U.S.A. ;
[3]Finnish Cancer Registry, Institute for Statistical and Epidemiological Cancer Research, Helsinki, Finland ;
[4]Finnish Cancer Registry, - Institute for Statistical and Epidemiological Cancer Research, Helsinki, Finland ;
[5]National Institute for Health and Welfare, Information Services Department, Helsinki, Finland ;
[6]University of Helsinki- Helsinki University Hospital, Department of Obstetrics and Gynecology, Helsinki, Finland

Study question: Does the probability of a live birth after ovulation induction (OI) and assisted reproductive technology (ART) with autologous oocytes differ in female cancer survivors compared to siblings?
Summary answer: The probability of a live birth was similar in female cancer survivors and siblings after OI, as well as ART treatments.
What is known already: As survival rates in early onset cancer survivors increase, reaching 80% in Western Europe, this comes at a cost of a risk for adverse late effects. For female cancer survivors these include diminished gonadal function and infertility, which have been reported major concerns for survivors and their family. There are, however, only a few studies available on fertility treatments and following live birth rates in female early onset cancer survivors. In one of them the likelihood of a live birth was reduced after ART with autologous oocytes in survivors compared to siblings.
Study design, size, duration: In this retrospective, register-based study, data from Finnish registers on cancer, birth and prescribed medications were merged to identify 9,092 female cancer survivors (diagnosed with cancer between 1953 and 2012 at the age of 0-39 years) and 10,030 female siblings of survivors.
Participants/materials, setting, methods: Fertility drug purchases in cancer survivors and siblings in 1993-2012, at the age of 20-41 years, were identified from the Reimbursement Register on Prescribed Medicines and live births from the Medical Birth Register. A binomial regression model with log-link function was used to calculate risk ratio (RR) for live births after fertility treatments in survivors and siblings. Fertility drugs were sub-classified into OI and ARTs and we adjusted for attained age and calendar time.
Main results and the role of chance: All in all, 198 (2.18 %) survivors and 269 (2.68 %) siblings were prescribed fertility drugs for OI, whereas for ART treatments the numbers were 173 (1.87 %) survivors and 215 (2.14 %) siblings. The cumulative live birth rate 22 to 48 weeks after fertility drug purchase was 9.1% (OI) and 14.7% (ART) among survivors and 8.2% (OI) and 14.4% (ART) among siblings. After adjusting for attained age and time period of fertility treatment, the probability of a live birth 22 to 48 weeks after fertility drug purchase was similar for survivors and siblings with OI (RR 1.07, 95% CI 0.64-1.78) and ART (RR 1.05,95% CI 0.72-1.52). The probability of a live birth occurring over 48 weeks after fertility drug purchase was also similar among survivors and siblings, for OI as well as for ART-treatments.
Limitations, reasons for caution: In this study, information on the indication for fertility treatment was unavailable and only fertility treatments with autologous oocytes were included. For live births occurring over 48 weeks after the fertility treatment, it is impossible to conclude whether they were the result of a spontaneous pregnancy or a fertility treatment.
Wider implications of the findings: For those cancer survivors, who were offered and opted for fertility treatments, the probability of live birth after OI or ART was similar compared to siblings. Today, personalized oncofertility counselling before and after gonadotoxic cancer treatments is considered standard care. This includes an evaluation of the fertility before planning pregnancy.
Trial registration number: Not applicable

POSTER VIEWING SESSION
REPRODUCTIVE SURGERY

P-747 Laparoscopic ovarian drilling for ovulation induction in women with anovulatory polycystic ovary syndrome – a cochrane review

E. Bordewijk[1], K.Y.B. Ng[2], L. Rakic[1], B.W.J. Mol[3], J. Brown[4], T.J. Crawford[4], M. Van Wely[1]

[1]Amsterdam UMC- University of Amsterdam, Center for Reproductive Medicine, Amsterdam, The Netherlands ;
[2]University of Southampton, School of Human Development and Health, Southampton, United Kingdom ;
[3]Monash University, Department of Obstetrics and Gynaecology, Clayton, Australia ;
[4]The University of Auckland, Liggins Institute, Auckland, New Zealand

Study question: To evaluate the effectiveness and safety of laparoscopic ovarian drilling (LOD) for women with anovulatory PCOS and CC-resistance.
Summary answer: LOD may decrease live birth slightly when compared with medical ovulation induction alone.
What is known already: Polycystic ovary syndrome (PCOS) is a common condition affecting 8-13% of reproductive-aged women. Clomiphene citrate (CC) or letrozole are first line treatments. Women who are CC-resistant or do not respond on letrozole, can be treated with gonadotrophins or other medical ovulation induction agents. These medications are not always successful, can be time-consuming and can cause adverse events like multiple pregnancies. LOD is a surgical alternative to medical treatment. There are risks associated with surgery, such as complications from anaesthesia, infection, and adhesions. This review aimed to determine its benefits and risks.
Study design, size, duration: This is an Cochrane review. We searched the Cochrane Gynecology and Fertility Group trials register, CENTRAL, MEDLINE, Embase, PsycINFO, CINAHL and two trials registers in October 2019 for relevant randomised controlled trials (RCTs), and checked references and contacted study authors in the field to obtain additional data.
Participants/materials, setting, methods: Women with anovulatory PCOS who underwent LOD as secondary treatment, with or without medical ovulation induction versus medical ovulation induction alone were included. We used standard methodological procedures as recommended by Cochrane for our search, data extraction, and analyses. The primary outcomes were live birth and multiple pregnancy. Pregnancy, miscarriage, OHSS, ovulation, and costs were secondary outcomes. We conducted subgroup analyses for the different medical ovulation induction agents.
Main results and the role of chance: This review includes 41 RCTs. We found LOD may decrease live birth slightly when compared with medical ovulation induction alone (OR 0.75, 95% CI 0.59 to 0.95; 1264 women; 10 studies; I^2 = 0%, *low quality evidence*), but according to the sensitivity analysis restricted to only RCTs with low risk of selection bias there is uncertainty whether there is a difference between the treatments (OR 0.93, 95% CI 0.67 to 1.30; 675 women; 5 studies; I^2 = 0%, *low quality evidence*). In absolute terms this implies that if the chance of live birth following medical ovulation induction alone is 37%, the chance following LOD would be between 28% and 43%. For pregnancy data suggested little or no difference (OR 0.88, 95% CI 0.75 to 1.03, I^2 = 0%; *low quality evidence*).

LOD probably reduces multiple pregnancy (OR 0.30, 95% CI 0.15 to 0.59; 1519 women; 16 studies; I^2 = 0%; *moderate quality evidence*). There is uncertainty about the effect on miscarriage (OR 1.11, 95% CI 0.80 to 1.54; 2349 women; 22 studies; I^2 = 0%; *low quality evidence*)). LOD reduced OHSS (peto OR 0.24, 95% CI 0.07 to 0.86; 1262 women; 10 studies; I^2 = 0%; *low quality evidence*).
Limitations, reasons for caution: The evidence was of very low to moderate quality: the main limitation was poor reporting of study methods. The main reasons for downgrading evidence were lack of details to be able to judge risk of bias (randomisation and allocation concealment) and lack of blinding.
Wider implications of the findings: LOD is an effective alternative to medical treatment, but more evidence is required to determine the impact of

LOD on live birth in women with anovulatory PCOS and CC-resistance compared with medical ovulation induction alone. Future RCTs are advised to include adverse effects, cost analyses and consumer satisfaction.
Trial registration number: not applicable

P-748 Intrauterine infusion of platelet-rich plasma after hysteroscopic adhesiolysis is a new treatment method for patients with intrauterine adhesions after hysteroscopy

J. Peng[1], M. Li[1], Z. Zeng[1], H. Zeng[1], J. Huang[1], X. Liang[1]
[1]The Sixth Affiliated Hospital of Sun Yat-sen University, Reproductive Medicine Research Center, Guangzhou, China

Study question: To evaluate the efficacy of an intrauterine infusion of platelet-rich plasma for the prevention of recurrent adhesions and the promotion of endometrial repair after hysteroscopy in patients with intrauterine adhesions.
Summary answer: Intrauterine infusion of PRP is a new treatment method in the management of patients with IUAs after hysteroscopic adhesiolysis
What is known already: Intrauterine balloon is one of the most important methods to prevent recurrent IUAs.
Study design, size, duration: This is a retrospective study, and three groups from 2018 to 2019 were included in this study: (1) intrauterine infusion of PRP after the first operations (group A);(2) intrauterine balloon after the first operations (group B) ;(3) both intrauterine infusion of PRP and intrauterine balloon after the first operations (group C). All patients were with severe or moderate IUAs.
Participants/materials, setting, methods: Thirty-eight patients in group A, thirty-two patients in group B and twenty-four patients in group C were included. The second-look hysteroscopy and the third-look hysteroscopy were performed one week after the first operation and during the next menstrual cycle respectively. AFS scoring system was used to judge the grade of the IUAs. Embryo transfer would be performed and serum β-HCG would be tested 12 or 14 days later.
Main results and the role of chance: In our study, 28 patients in group A, 22 patients in group B and 20 patients in group C received the third-look hysteroscopy. The mean AFS score decreased from 7.93±1.60 in the first hysteroscopy to 2.75±1.40 in the third hysteroscopy in group A, from 8.09±1.86 to 3.18±1.75 in group B, and from 8.20±1.63 to 3.05±1.63 in group C, respectively. No significant differences were found among these groups neither in the first operations (P>0.05) nor in the last operations (P>0.05) . The pregnancy rates were 40.0% (6 patients with positive β-HCG among 15 patients) in group A, 38.9% (7 patients with positive β-HCG among 18 patients) in group B and 33.3% (3 patients with positive β-HCG among 9 patients) in group C (P>0.05).
Limitations, reasons for caution: This is a retrospective study, and the conclusion of the study should be proved by RCTs.
Wider implications of the findings: Intrauterine infusion of PRP is a new treatment method for IUAs and it can be a substitute for intrauterine balloon.
Trial registration number: not applicable

P-749 Tubal infertility in advanced age- Are fallopian tubes still valuable to exist?

X. Yu[1], J. Guan[1]
[1]Reproductive Center, Beijing university People's hospital, beijing, China

Study question: We explored the optimal management for tubal infertile patients in advanced age with low reproductive prognosis
Summary answer: IVF compared with surgery demonstrates higher ongoing pregnancy rate in the setting of three or more follicles, especially for those younger than 40 years.
What is known already: Women of advanced age may have mild mechanical tubal factors which are more prevalent as age increases, and the co-exist reason for their infertility is reduced oocyte quantity and quality.
Study design, size, duration: Women (≥35 years old) with diminished ovarian reserve parameters who underwent IVF or tubal repair surgery from September 2016 and September 2017 was followed prospectively 12 months to determine the proportions of women undergoing the two procedure who became pregnant and who had ongoing pregnancy live.
Participants/materials, setting, methods: Ongoing pregnancy, clinical pregnancy, miscarriage and ectopic pregnancy rates per couple were compared for patients with IVF (n=95) versus tubal operation (n =39), and were stratified based on the female age and number of follicles triggered in IVF cycle.
Main results and the role of chance: Ongoing pregnancy rate per couple after IVF or tubal laparoscopy were similar (25.3 % vs.12.8 %, respectively). Ongoing pregnancy rates for patients aged 35-40 were significantly higher in IVF treatment compared with surgery when at least 3 follicles were present (46.7 % IVF vs.11.8 % surgery, $P<0.01$). No benefit was gained by pursuing IVF in the setting of one or two follicles. In patients >40 years, once again, there was little benefit gained when proceeding IVF as opposed to tubal surgery.
Limitations, reasons for caution: The limitation of this study is its retrospective nature. With extremely low pregnancy rates in tubal infertile patients with advancing age, a prospective study could be difficult for the large size of samples.
Wider implications of the findings: Tubal stage, as the important factor in the prognosis for success after surgical repair should be considered.
Patients with severe tubal pathologies and low ovarian response are urged to proceed IVF.
Trial registration number: no

P-750 Uterine rupture during pregnancy and succeeding fertility: The URIDA (uterine rupture international data acquisition) study

I. Kosmas[1], A. Tinelli[2], J. Carugno[3], O. Mynbaev[4], A. Malvasi[5], A.S. Laganà , for the URIDA study group[6]
[1]Ioannina State General Hospital G. Chatzikosta, Department of Obstetrics and Gynecology, Ioannina, Greece ;
[2]Vito Fazzi Hospital, Department of Obstetrics & Gynecology, Lecce, Italy ;
[3]University of Miami- Miller School of Medicine, Department of Obstetrics and Gynecology, Miami, U.S.A. ;
[4]Moscow Institute of Physics & Technology State University, Department of Obstetrics and Gynecology, Moscow, Russia C.I.S. ;
[5]GVM Care & Research Santa Maria Hospital, Department of Obstetrics and Gynecology, Bari, Italy ;
[6]"Filippo Del Ponte" Hospital- University of Insubria, Department of Obstetrics and Gynecology, Varese, Italy

Study question: The aim of this study is to specify the peripartum outcomes of patients with uterine rupture (UR) during pregnancy, and to estimate outcomes of subsequent pregnancies.
Summary answer: UR is a catastrophic event for mother and fetus, if the early diagnosis is missed. Otherwise, the outcomes are satisfactory even for a future pregnancy.
What is known already: Uterine rupture may take place in a healthy uterus, as in uterus that had undergone previous surgery, like myomectomy, laparoscopic salpingectomy and cesarean section (CS). This happens at 2nd and 3rd trimester of pregnancy. No data exist, except case reports about future fertility outcomes.
Study design, size, duration: This is a multi-center retrospective analysis. 270 women with complete rupture that took place at least in the last 15 years and followed for at least 5 years, was included in the analysis. Patients without a complete uterine rupture in pregnancy have been excluded.
Participants/materials, setting, methods: Women from 18 to 50 years old that diagnosed for uterine rupture during pregnancy, at 14 large gynecological centers through the world, were included. Data have been extracted and sent to the principal investigator. An independent statistician has examined the data. Demographic information, surgical history, symptoms and postoperative outcome for mother and the neonate has been recorded. Collection of the data has been performed in one year
Main results and the role of chance: 36 women had no history of surgery. Most recorded surgeries were a previous Cesarean Section (n = 113), abdominal (n = 10) or laparoscopic myomectomy (n = 9). Certain patients (n=5) had a second surgery along with the first one.

Subserosal fibroids on 25 patients with uterine rupture and 62 fibroids on the lower uterine segment are the most frequent fibroids operated. A rupture took place after resectoscopic myomectomy, in just 159 days of pregnancy. In laparotomic and laparoscopic myomectomy, rupture took place at 253 ± 52.35 and

251.22 ± 52.9 days respectively, and for patients with no previous surgery at 257.69 ± 43.15 days. Overall mean time was at 37, 32 ± 5, 09 weeks of pregnancy.

Many pregnancies delivered after 260 days (37 ± 14 weeks) of pregnancy. The birth weight was at 3106, 4 ± 653, 75 gr.

174 pregnancies conceived after uterine rupture, spontaneously in 169 women and in 5 by assisted reproductive techniques (ART). No data existed for 16 of them. 142 women delivered by CS, 13 delivered vaginally, 2 needed a vacuum extractor. The indications for CSs was twin pregnancy, cephalopelvic disproportion, emergency and placenta abruption.

Limitations, reasons for caution: This is a retrospective study, but from the other side it is a large multi-center investigation (one of the largest retrospective studies performed until now),that took place at hospital environment and by specialized personnel. The centers involved, were widespread through the world thus increasing the accuracy of the study.

Wider implications of the findings: Fertility preservation after UR is feasible with many patients to conceive spontaneously or by ART. Most of them had a CS, but a small number of them attempted and succeeded a vaginal delivery. Delivery time and birth weight in this pregnancy seems not to be affected from the previous rupture.

Trial registration number: NCT03576950

P-751 Usefulness of office hysteroscopy (OH) in patients with recurrent implantation failure (RIF)

G. Botti[1], L. Solari[1], C. Nazario[1], C. Morente[1], M.E. Mackey[2]

[1]PROAR, Videoendoscopy, Rosario, Argentina ;
[2]PROAR, Bioestatistical, Rosario, Argentina

Study question: Is OH a useful tool in the evaluation of uterine cavity in RIF patients? Does it improve the pregnancy rate in subsequent IVF/ICSI cycles?

Summary answer: OH is a useful, safe, fast tool in trained hands and well tolerated in RIF patients, with good pregnancy rates in subsequent IVF/ICSI cycles

What is known already: The success rate of IVF/ICSI remains low and many women must undergo multiple treatment cycles to achieve a pregnancy.. Several studies suggested OH could improve outcomes in women with RIF. TROPHY trial showed that OH before IVF/ICSI in women with RIF doesn´t improve the live birth rate, however in the literature there is some evidence of benefit from OH in this kind of patients showing increased pregnancy rates in subsequent IVF/ICSI cycles, both in those with normal and abnormal hysteroscopic findings.

Study design, size, duration: This is a cohort retrospective study

We performed OH in 84 RIF patients from july 2015 to june 2019

We define RIF as patients with two or more transfers of one blastocyst or two 72 hrs embryos.

A new embryo transfer cycle was done within six months afther OH.

Participants/materials, setting, methods: The procedures were done in PROAR Videoendoscopic Department, by the same operator (LS) in a vaginoscopic approach without anesthesia. We used a Bettocchi set and saline solution as a distention media.

All procedures were video recordered.

An endometrial biopsy was taken with a Pipelle cannula for anatomo-pathologic study.

OH was performed in early folicular phase or at any moment of the cycle in patients using oral contaceptives. The mean time of the procedure was 4,25 minutes (range: 2.20-8.45)

Main results and the role of chance: Four out of 84 OH were discontinued because of cervical stenosis.

In the remaining 80 patients we found 53 normal cavities (66%) and 27 abnormal (34%): 7 sinequiae, 4 adenomyosis, 2 polyps and 14 chronic endometritis (CE) confirmed with the presence of plasmocytes in the endometrial biopsy.

All patients with CE were treated with doxycycline (200mg daily for 14 days). We confirmed healing with a new biopsy in the next cycle.

Only one patient had a complication (vaso-vagal syndrome)

Pregnacy rates after OH were: 43% in normal cavities (23/53) and 52% in abnormal cavities (14/27).

Pregnancy rates in patients with pathologic findings were 28% in sinequiae (2/7), 25% in adenomyosis (1/4), 50% in polyps (1/2) and 71% in CE (10/14)

Limitations, reasons for caution: This is a retrospective study with a low number of cases.

Table 1: Diagnosis and pregnancy rates after OH

	PATIENTS	PREGNANCIES	PREGNANCY RATE	IC 95%
	n %	n	%	
NORMAL	53 66	23	43	(27.9-57.7)
ABNORMAL	27 34	14	52	(31.9-71.3)
- CE	14	10	71	(41.9-91.6)
- OTHERS	13	4	31	(9.1-61.4)
TOTAL	80	37	46	

CE was diagnosed with an anatomo-pathological criteria (plasmacytes presence in the endometrial biopsy sample). A more accurate method like flow cytometry analysis could improve CE diagnosis.

Wider implications of the findings: After OH we obtained high pregnancy rates in patients with CE and antibiotic treatment and also in patients with normal hysteroscopic findings.

Randomized controlled trials are needed before it routine use in general subfertile or RIF patients can be recommended

Trial registration number: Not applicable

P-752 what factors could predict recovery of serum anti-Mullerian hormon levels within 6 months after laparoscopic cystectomy for ovarian endometriomas

D. DAT[1], N. Ha[2], T. Phan[1], T. Anh duc[3], N.K. Toan[1]

[1]Hanoi Obstetrics and Gynecology Hospital, Gynecology Department, Hanoi, Vietnam ;
[2]Hanoi Obstetrics and Gynecology Hospital, ART center, Hanoi, Vietnam ;
[3]Hanoi Obstetrics and Gynecology Hospital, Obstetrics Department, Hanoi, Vietnam

Study question: This study aims to investigate the predictive factors that may be involved in the recovery of serum anti-mullerian hormone (AMH) levels within 6 months after laparoscopic cystectomy for ovarian endometriomas.

Summary answer: The decrease of AMH can recover after 6 months, the rate of reduction 1 month post-surgical AMH levels might predict AMH levels recovery.

What is known already: Study design, size, duration: A prospective longitudinal study was done with 104 patients undergoing laparoscopic cystectomy for unilateral endometriomas (n=77) and bilateral endometriomas (n=27). Serum AMH level was measured before surgery, at 1 month and at 6 months after surgery. Patients with serum AMH levels at 6-months higher than at 1-month after surgery were considered recovery. Many factors were assessed to find out the association with recovery of serum AMH level.

Participants/materials, setting, methods: A prospective longitudinal study

Main results and the role of chance: 52 patients (50%) showed higher AMH levels at 6 months than at 1 month after surgery (increase group) and 52 patients (50%) showed lower AMH levels (decrease group). Many factors as age of patients, the number side of ovary with endometriomas, size of endometriomas, ASRM score, duration of surgery, pre-surgical AMH level and rate of reduction AMH level (rAMH) at 1 month after surgery were analyzed and we found only statistically significant difference in rAMH at 1 month after surgery between two groups. The optimal cut-off point of rAMH at 1month for predicting AMH levels recover at 6 months after surgery was 52.77% in the patients undergoing unilateral cystectomy, 84.54% in the patients undergoing bilateral cystectomy.

Limitations, reasons for caution: no limitation

Wider implications of the findings: in the literature, there were many research in this field but still controversy

Trial registration number: not applicable

P-753 Post Implantation failure diagnostic Hysteroscopy with Endometrial Fundus Incision (FEI) might improve pregnancy outcome in oocyte recipients

E. Papanikolaou, MD- PhD[1], R. Najdecki[1], F. Chouliara[1], P. Tatsi[1], T. Chartomatsidou[1], F. Pakaki[1], S. Stamataki[1], C. Ioakeimidou[1], A. Timologou[1], C. Vlachou[1], A. Diamantopoulos[1], A. Virgiliou[2], P. Kaminski[3], A. Athanasiadis[2], E. Timotheou[1]

[1]Assisting Nature- Center of Reproduction & Genetics, Assisting Nature, Thessaloniki, Greece ;
[2]Aristotle University of Thessaloniki, 3rd Department Ob Gyn, Thessaloniki, Greece ;
[3]Military Institute of Medicine, Ob Gyn, Warsaw, Poland

Study question: To investigate whether hysteroscopic evaluation of the uterine cavity post implantation failure and FEI can improve pregnancy outcome in oocyte recipients.
Summary answer: Live Birth rate is higher in oocyte recipients, undergoing diagnostic Hysteroscopy compared to those who do not, after implantation failure, although not statistically significant.
What is known already: Implantation failure in oocyte recipients is devastating as the majority of them have previously undergone several failed IVF procedures, and therefore a new failure is difficult to be accepted. Appropriate evaluation of the uterine cavity is then of paramount importance and some advocate uterine scratching even in the presence of normal cavity.
Study design, size, duration: Between 2014-2019, 218 egg recipients underwent egg donation without hysteroscopy. Those who did not achieve live birth and had surplus embryos cryopreserved had a second chance by transferring in a subsequent frozen cycle these embryos. These patients were divided in two groups; **Hysteroscopy group (n=33)**: women who underwent hysteroscopy (Storz Bettocchi 5mm) prior to the 2nd embryo transfer (ET) and **Non- Hysteroscopy group (n=59)**: women who did not undergo hysteroscopy prior to the 2nd ET.
Participants/materials, setting, methods: The age of the studied oocyte recipients ranged from 35-50 years old. The rate of those who did not achieve a live birth at the 1st ET was 57.8% (n=126) and ninety-two of them had a second frozen-thawed ET. These patients underwent double blastocyst ET, unless only one cryopreserved blastocyst was still available. Women who had hysteroscopy before the 2nd ET all underwent FEI with endoscopic scissor irrespectively of the presence of pathology or not.
Main results and the role of chance: Among women of the Hysteroscopy group 20 were diagnosed with U2a (partial septate, arcuate uterus), whereas 13 were diagnosed with normal uterine cavity. The rest 59 recipients of the Non- Hysteroscopy group underwent a 2nd ET without hysteroscopy first. Live Birth rate was higher in the hysteroscopy group at a rate of 39,4% (n=13/33), compared to the non-hysteroscopy group 27,1% (n=16/59), although not statistically significant (p=0.087). Our current results with oocyte recipients indicate that once hysteroscopy is performed after implantation failure, a significant proportion of arcuate uterus is diagnosed and thus treated and second, that pregnancy outcome is improved after FEI irrespectively to the presence of arcuate uterus (U2a) or not (U0) with endoscopic scissor can improve pregnancy outcome
Limitations, reasons for caution: A larger sample size in both groups should provide more solid evidence regarding the impact of diagnostic hysteroscopy and FEI on the pregnancy outcome. Moreover, the interval time between the hysteroscopy and the subsequent ET, as well as the embryo quality of the 2nd choice embryos, should also be considered.
Wider implications of the findings: Although hysteroscopy with or without uterine scratching before embryo transfer, has not been universally accepted, clinical results indicate a different reality. The results of the current study indicate a potential correlation between FEI and pregnancy outcome. Further studies are required to confirm the above findings in larger groups.
Trial registration number: N/A

POSTER VIEWING SESSION
SAFETY AND QUALITY OF ART THERAPIES

P-754 Interventional ultrasound : standardisation of ultrasound guided Embryo Transfer (ET) in Medically Assisted Reproduction (MAR) Treatment

C. Panayotidis[1], O. Amototsero[2], N. Vermeulen[3], A. D'Angelo[4]

[1]Gynaecology, Isle of Wight, Isle of Wight, United Kingdom ;
[2]Cardiff University, University Hospital of Wales, Cardiff, United Kingdom ;
[3]ESHRE, Central office, Brussels, Belgium ;
[4]Wales Fertility Institute, University Hospital of Wales-, Cardiff, United Kingdom

Study question: Identify what could be the standards for USS guided ET in MAR
Summary answer: Proposed standards of practice that the ESHRE Ultrasound working group could consider for the developing good clinical practice on how to perform Ultrasound guided ET
What is known already: There are not many papers evaluating the technical aspects of ultrasound guided ET. There is a striking heterogeneity on ET techniques and particular on the role of ultrasound settings during the procedure.
Study design, size, duration: The study design included two steps: search for existing evidence through literature search and a Delphi method survey. The Delphi survey was carried out among 12 expert specialists on embryo transfer technique , using a 73 questions questionnaire completed in two rounds achieving a consensus for recommendations on ET practice.
Participants/materials, setting, methods: Experts in MAR were selected from a database of experts created by ESHRE as a result of a survey previously circulated. Delphi survey method was used for 2 round survey fousing on the following area: patient set up , ET preparation materials, ultrasound settings, ultrasound ET technique, prevention of complications and assessment of effective ET performanceLiterature review of relevant papers with emphasis on the ET technique and optimal ultrasound settings during the ET were retrieved.
Main results and the role of chance: Recommendations on practice were formulated based on the literature review and the expert opinions. This first consensus of the expert group included recommendation on patient set up, ET preparation materials, ultrasound settings, ultrasound ET technique, prevention of complications and assessment of effective ET performance. The majority (>55%) of experts use: trans-abdominal ultrasound with the help of a trained assistant, clean the cervical plug using cotton swabs, use mostly soft catheters and if need they propose stylet catheters as an alternative. Most of the experts consider factors that negatively influence the success of the ET if intrauterine fluid is visualised prior ET, blood clot visualised during ET, uterine malformation, uterine caesarean section scar, excessive cervical mucus abnormal uterine cavity length. Experts consider complications to be associated with ET when cervical trauma occurs and least as a result of an infection. The use of bubbles to mark the embryo during the ET was predominant in their practice. Variation on ultrasound settings are analysed in regards image optimisation. These proposed standards on practice can now be presented and discussed by ESHRE aiming to be implemented in clinical practice through the publication of a clinical guideline.
Limitations, reasons for caution: Consider limitations on Delphi survey technique, consensus of expert opinions can be a starting point to discuss further what can represent the best ET practice in 2020. Further Survey rounds may be needed and the established ESHRE methodology for guideline production should be applied
Wider implications of the findings: This is the first European survey of ESHRE on USS guided ET technique aiming to formulate recommendations for good clinical practice on how to perform ET with optimal use of ultrasound technology. These recommendations will produce clinical standards in MAR enhancing the effectiveness of ET in increasing clinical pregnancy rates.
Trial registration number: 0

P-755 Do medical assisted reproduction and female infertility impact the risk of imprinting disorders in singletons? A longitudinal national French study

P. Fauque[1,2], G. Viot[3], D. Aviva[4], F. Pessione[5], A.W.C.H. ABM-working group[6]

[1]Centre Hospitalo-Universitaire, Laboratoire de Biologie de la Reproduction-CECOS, Dijon, France ;
[2]INSERM, Unit 1231, Dijon, France ;
[3]Clinique de La Muette, Unité de Génétique, Paris, France ;
[4]Centre Hospitalo-Universitaire, Centre d'assistance médicale à la procréation, Amiens, France ;
[5]Agence de la biomédecine, Direction médicale et scientifique, Paris, France ;
[6]Agence de la biomédecine, Groupe de travail: suivi des femmes et des enfants en AMP, Paris, France

Study question: Do medical assisted reproduction and female infertility (i.e. endometriosis, polycystic ovary syndrome [PCOS] and primary ovarian insufficiency [POI]) increase the risk of imprinting disorders in singletons?
Summary answer: The risk of imprinting disorders was increased in children conceived after fresh embryo transfers or from mothers with endometriosis, especially for neonatal diabetes mellitus (NDM).
What is known already: Epidemiological studies suggest that singletons born from assisted reproductive technologies (ART) have a higher risk of adverse perinatal outcomes, specifically for imprinting disorders. Because ART manipulations and processes take place at times when epigenetic reprogramming and imprinting are occurring (i.e. during female gametogenesis and preimplantation embryo development), there is concern that ART can induce adverse epigenetic effects and affect the establishment/maintenance of genomic imprints. However, little is currently known about the risk of imprinting defects according to the type of ART or the type of underlying female infertility.
Study design, size, duration: Using data from the French National System database (SNDS), we conducted a comparative analysis of all singleton births (deliveries ≥22 weeks of gestation and/or >500g of birthweight) that occurred in France over a 5-year period (2013-2017) resulting from fresh embryo or frozen embryo transfers (fresh-ET or FET from IVF/ICSI cycles), intrauterine insemination (IUI) and natural conceptions (NC). Data was available for this cohort of children at least up to early childhood (mean 2.5 years old).
Participants/materials, setting, methods: A total of 3,501,496 singleton births were included (including 20,218 from IUI, 45,303 from fresh-ET; 18,885 from FET). Data were extracted from national health databases. We monitored syndromes/diseases involving imprinted genes: Beckwith-Wiedemann's, Silver-Russell's, Prader-Willi's and Angelman's syndromes, pseudohypoparathyroidism, NDM, syndromes affecting the imprinted region of chromosome 14q32, neuroblastoma and retinoblastoma. Univariate and multivariate analyses were performed with multiple logistic regression models to analyse the effect of ART conception and the effect of female infertility (endometriosis/PCOS/POI).
Main results and the role of chance: In our cohort of children, the prevalence of imprinting-related diseases was 0.10% after NC, 0.16% after fresh-ET, 0.12% after FET, and 0.11% after IUI. Compared with infants conceived naturally, children born after fresh-ET had a significantly higher prevalence of imprinting diseases, with an aOR of 1.43 [95%CI 1.13–1.81, p=0.003]. In particular, in univariate analysis, we observed a significant increase in the number of cases of NDM in the fresh-ET group compared with the NC group. This increased risk of NDM was confirmed in multivariate analysis (1.96 aOR [95%CI 1.43–2.70], p<0.0001), even when the type of female infertility was taken into account (1.58 aOR [95%CI 1.13–2.21], p=0.008). The overall risk of imprinting disorders was similar in the IUI group, the FET group and the NC group, as well as for specific imprinting-related diseases. There was an overall independent increase in risk of imprinting diseases for children with mothers diagnosed with endometriosis (1.38 aOR [95%CI 1.06–1.80], p=0.017). Specifically, whatever the mode of conception, the NDM risk tended to be higher for children conceived by women with endometriosis (1.47 aOR [95%CI 1.00–2.16], p=0.051). On the contrary, the children of women with POI and PCOS had no particular significant epigenetic risk.
Limitations, reasons for caution: For some extremely rare syndromes, the sample size may be too small reliably conclude that there was no difference between groups. The underlying molecular mechanisms were not known.
Wider implications of the findings: The increased perturbations in genomic imprinting could be caused by controlled ovarian hyperstimulation (and potentially endometriosis) through the impairment of endometrial receptivity and placentation, leading to epigenetic feto-placental changes. These novel findings highlight the importance of considering the mode/process of conception and the infertility context in children health follow-up.
Trial registration number: Not applicable

P-756 Preparing the endometrium with natural cycles protects patients from an increased risk of adverse obstetric and neonatal outcomes after frozen-thawed embryo transfer

C. Li[1], X. Jing-Jing[1], W. Yan-Ting[1], H. He-Feng[1]

[1]International Peace Maternity and Child Health Hospital- School of Medicine-Shanghai Jiao Tong University, Department of Reproductive Medicine, Shanghai, China

Study question: To explore the association between different endometrial preparation protocols and adverse obstetric and perinatal complications in patients after frozen embryo transfer (FET).
Summary answer: Preparing the endometrium with natural cycles could prevent the increased risk of pregnancy-related complications and the increased risk of adverse neonatal outcomes after FET.
What is known already: Previous studies have focused on pregnancy outcomes after FET performed using different endometrial preparation protocols. Few studies in the literature have evaluated the effect of endometrial preparation on pregnancy-related complications, especially in ovarian stimulation protocols.
Study design, size, duration: This retrospective cohort study included all FET cycles (n=12,950) performed between 2010 and 2017 in our hospital.
Participants/materials, setting, methods: All FET cycles were categorized into three groups according to the endometrial preparation protocol. Pregnancy-related complications and subsequent neonatal outcomes were compared among groups using multilevel logistic regression.
Main results and the role of chance: Among all 12,951 FET cycles, the live birth rate was slightly lower for HRT cycles than for natural cycles (28.15 vs. 31.16%, p<0.001). The pregnancy loss rate was significantly higher in ovarian stimulation (OS) or hormone replacement therapy (HRT) cycles than in natural cycles (NC vs. OS vs. HRT: 10.89% vs. 16.44% vs. 17.14%). Among women with live birth, preparing the endometrium using OS or HRT protocols increased the risk of preeclampsia, and ICP was found in both singleton and multiple deliveries. Additionally, OS and HRT protocols increased the risk of LBW and SGA in both singletons and multiples after FET.
Limitations, reasons for caution: As a hospital-based study, several confounders related to medical procedures have been taken into consideration. However, it is not possible to rule out unknown confounders. Bias induced by patients' favor for different endometrial preparation protocols is another inevitable limitation due to the retrospective design.
Wider implications of the findings: Caution is warranted when using OS or HRT protocols due to the increased risk of gestational hypertension, preeclampsia, and ICP as well as the risk of LBW and SGA.
Trial registration number: Not applicable

P-757 Relationship between number of oocytes retrieved and multiple pregnancies

"Abstract withdrawn by the authors"

P-758 How to reduce risk of genetic disease in gamete donor children

H. Hjalgrim[1], S. Timshel[2]

[1]Amplexa, Amplexa Genetics, Odense, Denmark ;
[2]European Sperm Bank, Copenhagen, Denmark

Study question: Can we prevent the birth of donor children with autosomal recessive disorders by genetically matching the donor and recipient, prior to fertility treatment?
Summary answer: It is possible to reduce the risk of genetic diseases in gamete donor children by routinely offering genetic matching.
What is known already: Every individual carries a number of abnormal variants in genes associated with autosomal recessive diseases. Even if most sperm banks screen their donors for the most common of these, donor children are still born with autosomal recessive diseases. This will only happen if both donor and recipient carry variants in the same genes. Screening of gamete donors varies highly in both extent and quality of the genetic tests. Only a few sperm banks recommend genetic testing of the recipient and even fewer offer matching of donor and recipient.
Study design, size, duration: A case report and series study based on genetic reports of children born with autosomal recessive diseases from European Sperm Bank (ESB) donors January 1st, 2012- December 31st, 2019. Furthermore, results of all donor-recipient genetic matches performed for a consecutive 15 months period (September 1st, 2018 – December 31st, 2019).
Participants/materials, setting, methods: Genetic reports from ESB donor children with autosomal recessive diseases diagnosed in the mentioned period. Carrier status subsequently confirmed in donor.

Recipients of donor sperm from ESB are screened at Amplexa Genetics with GeneXmatch, a customized targeted NGS gene panel, including app. 400 autosomal recessive genes. The recipients are matched against similar genetic data from their chosen donor. A no-match is reported as such when donor and recipient carry variants in the same genes.

Main results and the role of chance: *Main results:*

During the observation period sperm from ESB donors gave rise to app. 30.000 reported births. ESB received genetic reports from 23 children born with documented autosomal recessive diseases, where the sperm donor subsequently proved to be a carrier of the variant in question. GeneXmatch has been an option from September 1st, 2018; 17% of matches were no-matches, prompting the recipient to choose another donor. If GeneXmatch had been an option from 2012, the birth of 17 of the 23 reported children with autosomal recessive disease could have been prevented.

Role of chance: In all the reported 23 cases, the donors were proved to be carriers of pathogenic variants found in the donor children. The number of no-matches would probably have been higher had the donors not been screened for and excluded if carriers of cystic fibrosis, congenital deafness and spinal muscular atrophy.

Limitations, reasons for caution: The findings represent a minimum – not all children born with a genetic disease are reported to the respective sperm banks. Furthermore, not all children with a congenital disease are offered genetic testing.

Wider implications of the findings: To reduce the number of children born with autosomal recessive disorders from gamete donors, genetic matching between donor and recipient should be offered routinely. Genepanels for this should be high quality, cover autosomal recessive disorders with carrier frequencies above 1/100, well recognized geno-phenotype correlation with high penetrance and early onset.

Trial registration number: not applicable

P-759 Associations between meteorological perturbation, ambient air pollution and outcomes during ART treatment

J. Liu[1], M. Zhao[2], H. Zhang[3], J. Zhao[1], H. Kong[1], M. Zhou[1], Y. Guan[1], T.C. Li[2], X. Wang[1], D.Y.L. Chan[2]

[1]Third Affiliated Hospital of Zhengzhou University, Reproductive Medicine Center, Zhengzhou, China ;
[2]Faculty of Medicine- The Chinese University of Hong Kong, Assisted Reproductive Technology Unit- Department of Obstetrics and Gynaecology-, Hong Kong, China ;
[3]School of Sun Yat-sen University, Data and Computer Science, Guangzhou, China

Study question: Are meteorological perturbation and air pollution related to the outcome of IVF?

Summary answer: The implantation and clinical pregnancy is affected by temperature perturbation, however, not affected by air pollution during ART treatment in the reported area.

What is known already: Meteorology may affect IVF pregnancy rate. Air pollution may reduce IVF pregnancy rates and live births and increase miscarriage rates. At the same time, the risk of adverse obstetrics may be increased. However, the impact of meteorological perturbation and air pollution on IVF treatment outcomes is inconclusive in a few published articles.

Study design, size, duration: Retrospective cohort study of 6967 fresh, autologous IVF cycles for the first time at the Reproductive Medicine Center of the Third Affiliated Hospital of Zhengzhou University was performed between 2015.10 and 2018.12.

Participants/materials, setting, methods: Meteorological and air pollutant data came from China's relevant official website (http://data.cma.cn/) (http://106.37.208.233:20035/). The exposure was divided into 4 periods: gonadotropin (Gn) injection to oocyte retrieval (OR) (P1), OR to embryo transfer(ET) (P2), one day after ET to ET +14 days (P3), Gn injection to ET +14 days (P4). Multivariate logistic regressions were used to estimate the association between interquartile increases in variables and reproductive outcomes. Adjust other parameters that affect IVF treatment outcomes.

Main results and the role of chance: The mean age and BMI were 31.52±4.92 years and 23.30±3.23kg/cm^2. Meteorological parameters included average surface temperature, daily highest surface temperature, daily lowest surface temperature, average pressure, average relative humidity, sunshine hours and average temperature. Air pollutant parameters included CO, NO2, OPM10, PM2.5 and SO2. The effect of daily lowest surface temperature on implantation and clinical pregnancy was confirmed by multivariate analysis. Compared with women exposed to Q1 of daily lowest surface temperature, women exposed to Q4 period had highest probability of implantation and clinical pregnancy rates during P1 (a OR 1.26, 95% CI 1.08–1.46 for implantation; a OR 1.26, 95% CI 1.08–1.47 for pregnancy) and P2 periods (a OR 1.25, 95% CI 1.07–1.45 for implantation; a OR 1.27, 95% CI 1.09–1.47 for pregnancy), followed by Q3(a OR 1.14 for implantation and 1.04 for pregnancy) and Q2 (a OR 1.03for implantation and 1.16 for pregnancy) during P1, followed by Q3(a OR 1.12 for implantation and 1.01 for pregnancy) and Q2 (a OR 1.02 for implantation and 1.11 for pregnancy) during P2. There was no significant correlation between clinical outcomes and air pollution variables.

Limitations, reasons for caution: Meteorology and ambient air pollution level in specific areas may not represent the actual level of air pollution that every woman is exposed to. There may be a correlation between climate and air pollution parameters, which may lead to biased results.

Wider implications of the findings: More accurate instruments are needed to timely measure the various meteorological and air pollution parameters of the patients' environment, in order to more accurately assess their impact on the outcome of IVF treatment.

Trial registration number: processing

P-760 Comparison of perinatal outcomes between day 5 and 6 blastocyst transfers in both fresh and frozen cycles

C. Cheung[1], J. Kasraie[1], I. Solis-Trapala[2], A. Sizer[1]

[1]Shrewsbury and Telford Hospital NHS Trust, Shropshire and Mid Wales Fertility Centre, Shrewsbury, United Kingdom ;
[2]Keele University, School of Primary- Community and Social Care, Staffordshire, United Kingdom

Study question: Does extended culture to day 6 (D6) blastocysts increase adverse neonatal outcomes in both fresh and frozen cycles compared to standard day 5 (D5) blastocysts?

Summary answer: The mean birthweight, gestation, congenital anomalies and other neonatal morbidity did not differ between D5 and 6 transfers in both fresh and frozen groups.

What is known already: Previous studies have demonstrated that the type of culture medium and duration of culture have impact on birth weights. Many studies have shown that blastocyst transfers are associated with higher birth weights when compared to cleavage stage embryos. There are also reports of increased risk of adverse neonatal outcomes such as preterm birth and congenital anomalies with prolonged culture. D6 blastocysts are slower developing and have also spent longer in culture medium. There are very few studies, with controversial results, examining whether D6 blastocysts are associated with adverse neonatal outcomes when compared against normally developing D5 blastocysts.

Study design, size, duration: This is a retrospective cohort study conducted in a single unit. 637 fresh blastocyst transfer cycles (481 D5 and 156 D6 cycles) and 446 frozen cycles (291 D5 and 155 D6 cycles) from January 2013 to December 2018 included. Perinatal outcomes from singleton births were compared between D5 and 6 groups. Fresh and frozen cycles were analysed separately. 223 live births from the fresh group and 79 from the frozen group were available for analysis.

Participants/materials, setting, methods: In the fresh treatment group, all patients underwent fresh autologous cycles and fulfilled our unit's single blastocyst transfer criteria. All blastocysts in the frozen group were exclusively vitrified either from surplus embryos in a preceding fresh cycle or from a 'freeze all cycle'. D5 and 6 blastocysts were transferred after the same duration and dose of progesterone supplement. Mixed D5 and 6 transfers were excluded. Data were analysed using multiple logistic regression model.

Main results and the role of chance: In the fresh cycles, patients' age, BMI and number of previous treatment cycles between the D5 and 6 groups were similar. D5 transfers resulted in higher live birth rates compared to D6 group (D5=43.9%, D6=31.4%, $p<0.05$). Miscarriage, ectopic rates and multiple pregnancies resulting from monozygotic twinning were comparable.

184 live births from the D5 group were compared against 39 births from the D6 group. There were no significant differences in mean birthweight (D5= 3305g, D6= 3346g, P=0.65) and gestation (D5= 38.8, D6=38.9, P=0.82). The rates of large and small for gestational age, preterm births, congenital anomalies and stillbirths did not differ between the groups.

In the frozen cycles, there were no significant differences between the mean age at freeze and treatment, BMI, previous number of treatment cycles and number of blastocysts transferred. Live birth rates were not significantly different (D5=22.7%, D6=24.9%, p=0.77). Miscarriage, ectopic and multiple pregnancy rates were similar.

49 live births from D5 were compared against 30 births from D6 group. There were no significant differences in mean birthweight (D5=3533g, D6=3456g, p=0.59) and gestation (D5=38.8, D6=38.7 p=0.90). The rates of large and small for gestational age, preterm births, congenital anomalies and stillbirths did not differ between the groups.

Limitations, reasons for caution: A relatively small number of livebirths were analysed in our study, particularly from D6 groups. Some data were missing from patients who delivered out of area. Other potential factors such as maternal hypertension, pre-eclampsia and diabetes that can also influence birth outcomes were not taken into account in this study.

Wider implications of the findings: We demonstrated from our data that D6 blastocyst transfers did not result in an increased rate of adverse perinatal outcomes. Larger studies including day 7 transfers with long term follow up on children born from prolonged culture should be undertaken to clarify the safety of transferring D6 and D7 blastocysts.

Trial registration number: not applicable

P-761 Impact of endometriosis on maternal and perinatal morbidity in IVF versus spontaneous conception: a national 6-years observational study

S. Epelboin[1], R. Levy[2], M. Valentin[3], F. Pessione[4]

[1]Assistance Publique Hôpitaux de Paris Hôpital Bichat-Claude Bernard, Gynecology Obstetrics Reproductive Medecine, Paris, France ;
[2]Assistance Publique Hôpitaux de Paris Hôpital Tenon Université de Paris, Embryology, Paris, France ;
[3]Assistance Publique Hôpitaux de Paris Hôpital Bichat-Claude Bernard, Obsterics, Paris, France ;
[4]Agence de la Biomédecine, Epidemiology Biostatistics, Saint Denis, France

Study question: Which role of endometriosis and which additional risk of In Vitro Fertilisation(IVF) in maternal or fetal morbidity when comparing single deliveries of populations with/without endometriosis, after spontaneous or IVF conception?

Summary answer: There is an independent role of endometriosis in the increased adjusted risk of most maternal and perinatal morbidities in spontaneous deliveries, further increased by IVF

What is known already: Large observational studies identified that IVF pregnancies are associated with a significant risk of complications, even if single, as compared with pregnancies after spontaneous conception (SC) . These complications include preterm delivery, gestational diabetes, pre-eclampsia and placental anomalies, leading to increased fetal loss, intrauterine growth restriction, all events being a significant concern (Pinborg, 2013, Ombelet, 2016, Wang, 2016, Luke, 2017, Vermey, 2018). Moreover, several recent studies identified that compared with women without endometriosis, women with endometriosis had higher odds of maternal morbidities as hypertensive disorders, diabetes, placenta previa, placental abruption, preterm birth (Zullo, 2017, Lalali, 2018, Porpora,2020). Which respective responsibility?

Study design, size, duration: This is an observational national cohort study comparing maternal (MM) and perinatal (PM) morbidities in 3 groups of single pregnancies registered between 2013 and 2018 in France. The first group was SC without endometriosis (SC-non-endo), the second was SC with endometriosis (SC-endo), the third included IVF standard or using intracytoplasmic injection and fresh transfers with endometriosis (IVF-endo). Births were considered when > 22 weeks of gestation (WG), and term birth was defined > 37 WG.

Participants/materials, setting, methods: Pregnancies and deliveries were analyzed by extracting the Information Systems Medicalization Program (PMSI) French database. The main identified morbidity indicators were: venous thrombosis, gestational diabetes, hypertensive disorders (gestational hypertension or pre-eclampsia), placenta previa, placental abruption, premature birth, small for gestational age (SGA), major congenital malformations. The risks of MM and PM were estimated in multivariate analysis after adjustment for maternal age, smoking and obesity, primiparity and history of diabetes and hypertension.

Main results and the role of chance: The study included all 4,170,780 national single deliveries, among which 38035 (0.9%) with endometriosis. In the SC group, endometriosis was diagnosed in 31,101 mothers (0.76%). In the IVF group (55,947), endometriosis was diagnosed in 6,934 (12.4%).

Mean maternal ages were 30.0 (std=5.3), 31.7 (std=4.8) and 33.1 (std=4.0), for SC, SC-endo and IVF-endo groups.

Compared to control group, SC-endo and IVF-endo groups showed respectively lower rates for obesity (3.9% and 3.1% versus 4.9%), and smoking (3.7% and 2.4% vs 4.7%) (p< 0. 0001). Respective rates in SC-endo and IVF-endo were higher for primiparity (47.4% and 76.7% versus 39.6%), and history of hypertension (1.14% and 1% vs 0.85%). There was no difference for history of diabetes mellitus.

In SC group, endometriosis was associated with increased risks of venous thrombosis, hypertensive disorders, placenta previa, placenta abruption, premature birth (all p<.0001), congenital malformation (p= .049) and SGA (p<.0003) . In the endometriosis group, the comparison of highlighted aOR of SC-endo and IVF-endo populations, versus SC-non-endo , provided evidence that IVF techniques increased the risk of placenta previa (aOR 2.62 vs 6.51, p <.0001), premature birth (aOR 1.37 vs 1.92, p<.0001), and SGA (aOR 1.05 vs 1.25, p<.0001), respectively, No other significant difference was found (thombosis, diabetes...).

Limitations, reasons for caution: While the strength of this study lies in the number and completeness of subjects studied, its limitations are its register-based nature, not allowing to refine the risk evaluation, mainly according to the type of endometriosis (peritoneal, deep, associated adenomyosis), which can determine complications of variable importance (vascular, placentation).

Wider implications of the findings: These data demonstrating an independent role of endometriosis in the increased adjusted risk of most maternal and perinatal morbidities in spontaneous and IVF deliveries may improve the vigilance of practitioners in monitoring pregnancies when endometriosis, and be considered in the debate on the place of surgery for infertile endometriotic women.

Trial registration number: not applicable

P-762 Impact of subchorionic haematoma in early pregnancy on obstetric complications: A retrospective cohort study in women who had live births after frozen-thawed embryo transfer

O. Mochizuki[1], S. So[2], N. Murabayashi[2], W. Yamaguchi[1], N. Miyano[1], F. Tawara[1]

[1]Tawara IVF clinic, Reproductive medicine, Shizuoka, Japan ;
[2]Hamamatsu University School of Medicine, Department of Reproductive and Perinatal Medicine, Hamamatsu, Japan

Study question: We investigated whether subchorionic haematoma (SCH) in early pregnancy is involved in the development of obstetric complications in women who underwent frozen-thawed embryo transfer.

Summary answer: SCH in early pregnancy may contribute to the development of placenta previa and a clinically adherent placenta.

What is known already: SCH is known to contribute to abortion and premature birth. It has also been reported that taking aspirin during pregnancy contributes to the development of SCH. Pregnancy following assisted reproductive technology (ART) has been reported to have a high frequency of obstetric complications, but its association with the presence of SCH is not known.

Study design, size, duration: This study was approved by the Ethics Committee of Tawara IVF Clinic. Of the 5139 patients who underwent frozen -thawed embryo transfer between March 2015 and September 2018, 1426 women who had live births were included in this study.

Participants/materials, setting, methods: SCH was usually assessed at pregnancy weeks 7–9. Obstetric complications data were obtained from delivery hospital records. Multivariate analysis controlled for potential confounding effects: maternal age, body mass index, history of pregnancy and miscarriage, embryo stage at transfer, number of embryos transferred, use of assisted hatching, endometrial preparation method, aspirin use during pregnancy, and infertility cause (male infertility, polycystic ovary syndrome,

leiomyoma, endometrial polyps, adenomyosis, fallopian tube obstruction, endometriosis).

Main results and the role of chance: Of 1426 women who had live births, 343 women (24.1%) had SCH in early pregnancy. There was no difference in the frequency of SCH between the hormone replacement cycle and natural ovulatory cycle. There was also no difference in endometrial thickness (SCH group 9.9±1.8 mm vs. non-SCH group 10.0±1.7 mm) and aspirin use during pregnancy (7.6% vs. 9.4%). SCH was more frequent in women who had infertility due to endometrial polyps (SCH 9.4% vs. non-SCH 5.0%, p<0.001). Women with SCH in early pregnancy had a significantly higher risk of placenta previa (odds ratio (OR) 3.67 [1.21–11.60]) and a clinically adherent placenta (7.18 [3.04–18.41]) compared with that of non-SCH women. No differences were found in the development of hypertensive disorders of pregnancy (0.91 [0.49–1.60]), non-reassuring foetal status (1.56 [0.87–2.74]), foetal growth restriction (1.86 [0.61–5.24]), chorioamnionitis (2.42 [0.68–8.06]), premature rupture of the membranes (1.14 [0.65–1.95]), caesarean section (1.07 [0.75–1.51]), and preterm-delivery (1.24 [0.68–2.19]).

Limitations, reasons for caution: The size and location of the SCH was unknown. It is not known whether SCH was continuously present during pregnancy.

Wider implications of the findings: The group that developed SCH in early pregnancy had a significantly higher frequency of placenta previa and a clinically adherent placenta than that in the non-SCH group. Attention should be paid to the presence of SCH during early pregnancy after frozen -thawed embryo transfer.

Trial registration number: not applicable

P-763 Is the information on endometrial scratching provided by IVF clinics' websites biased?

V. Galiano[1,2], R. Orvieto[2,3], R. Machtinger[2,3], R. Nahum[2,3], E. Garzia[4], P. Sulpizio[4], A.M. Marconi[1], D. Seidman[2,3]

[1]ASST Santi Paolo e Carlo - San Paolo University Hospital, Department of Obstetrics and Gynecology, Milan, Italy ;
[2]The Chaim Sheba Medical Center, Infertility and IVF Unit - Department of Obstetrics and Gynecology, Ramat Gan, Israel ;
[3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel ;
[4]ASST Santi Paolo e Carlo - San Paolo University Hospital, Reproductive Medicine Unit, Milan, Italy

Study question: How accurate is the information about endometrial scratching presented on fertility clinics websites, considering the conflicting evidence on the effectiveness of this procedure?

Summary answer: The information about endometrial scratching available to the public from IVF-clinics' websites is often inaccurate.This could perpetuate false myths among infertile patients about the procedure.

What is known already: The impact of endometrial scratch on live birth rates remains inconclusive. Yet, this procedure is actively promoted by some fertility clinics as a proven means to improve IVF success rates, especially in couples with repeated implantation failure. Little information is currently available on the type and quality of information provided on IVF clinic's websites in regard to the merits of endometrial scratch.

Study design, size, duration: A systematic evaluation of fertility clinics websites**.** We developed a 10-criteria structured questionnaire to evaluate the quality of information about endometrial scratching available through the internet. The search and review were performed in January 2020 by a single investigator.

Participants/materials, setting, methods: We included in the study all websites of fertility clinics that presented in the first 30 pages of the Google.com search engine after typing "endometrial scratching" as the key word.

Main results and the role of chance: Our search identified 55 websites that all belonged to private IVF clinics. Almost half of the clinics were from the UK (23/55, 41.8%). Only 22 of 55 (40%) websites reported an accurate description of the procedure and how it is performed, and only 8 of 55 (14.5%) websites reported on the undetermined effectiveness of the procedure. Furthermore, 13 (23.6%) websites bluntly encouraged its use. The cost of the procedure was clearly displayed in only 5 (9.1%) cases and in only 7 (12.7%) websites a bibliography on the procedure was provided, mostly studies supporting the procedure. Less than half of the websites clearly noted (23/55, 41.8%) associated risks, such as infection, endometrial injury or uterine perforation. However, the possibility of pain during and after the scratch was often (34/55, 61.8%) described. Many of the reviewed websites were not updated, and data was often presented (20/55, 36.4%) with no date of entry. None of the websites reported the clinic's pregnancy rate following the procedure.

Limitations, reasons for caution: Our limited sample size may not be fully representative and was limited to English language websites.

Wider implications of the findings: Much of the information available to the public regarding new fertility enhancing "add-on" treatments is currently provided through fertility clinic's websites.ESHRE,as well as other leading professional organizations,should deliver better guidelines for fertility clinic websites, as there is an urgent need to improve the type and quality of information currently provided.

Trial registration number: Not applicable

P-764 Impact of frozen-thawed embryo transfers and hormone substitution on thrombotic risk markers

T. Dalsgaard[1], K. Kirkegaard[2], A.M. Hvas[3], U. Knudsen[1]

[1]Clinical Medicine, Gynaecology and Obstetrics, Horsens, Denmark ;
[2]Clinical Medicine, Gynaecology and Obstetrics, Aarhus, Denmark ;
[3]Clinical Medicine, Clinical Biochemistry, Aarhus, Denmark

Study question: Is there a difference in thrombotic risk markers between women receiving artificial cycle frozen embryo transfer (AC-FET) compared to natural cycle frozen embryo transfer (NC-FET)?

Summary answer: Women receiving AC-FET have an activated coagulation indicated by increased thrombin generation while being estrogen substituted. Such coagulation activation might induce an increased thromboembolic risk.

What is known already: Fertility treatment with frozen embryo transfer (FET) is used widely. Women treated with AC-FET receive high doses of estrogen in contrast to women treated with NC-FET. Estrogen substitution is likely to be associated with an increased risk of thromboembolism based on previous research on hormone replacement therapy in other contexts. Additionally, an increased risk of thrombosis has been shown in pregnant women following assisted reproductive technology treatment as compared to natural fertilization. However, the knowledge of how the coagulation is activated in women undergoing estrogen substitution during treatment with AC-FET remains unknown.

Study design, size, duration: Prospective cohort study of women receiving AC-FET with estrogen/progesterone substitution or NC-FET. Blood samples were obtained four times: 1) prior to hormone substitution (baseline), 2) confirmation of biochemical pregnancy, 3) gestational week 8 and 4) gestational week 13 (5 weeks after cessation of hormone substitution). Inclusion criteria: women aged >18 years assigned for FET. Exclusion criteria: egg donor recipients, known bleeding disorders, indication for thromboprophylaxis and treatment with anti-platelet medication or non-steroid-anti-inflammatory drugs.

Participants/materials, setting, methods: Women were recruited at the Department of Obstetrics and Gynaecology, Horsens Fertility Clinic, Denmark, August 2019 – June 2020. In total, 18 participants have been included: 10 women treated with AC-FET and 8 women receiving NC-FET. We examined alterations in surrogate markers of thromboembolic risk: primary outcome was the difference in thrombin generation expressed as endogenous thrombin potential before, during and after hormone stimulation. Secondary endpoint was platelet aggregation assessed by whole blood impedance aggregometry.

Main results and the role of chance: We found an increased thrombin generation after hormone substitution within the group treated with AC-FET compared to NC-FET indicated by a significantly shorter time to peak (3 ± 0.1 min versus 6.4 ± 0.4 min) ($p < 0.0001$), a significantly higher mean peak (329 ± 13 nM versus 259 ± 13 nM) ($p = 0.002$) and higher endogenous thrombin potential (1827 ± 480 nM min versus 1544 ± 131 nM min) ($p=0.02$). When compared to the NC-FET group, women receiving AC-FET had significantly higher thrombin generation shown by a shorter mean time to peak (5.6 ± 0.2 min versus 8.3 ± 0.7 min) ($p = 0.0006$), higher mean peak (329 ± 13 nM vs 174 ± 15 nM) ($p < 0.0001$) and a larger endogenous thrombin potential (1827 ± 160 nM min vs 1375 ± 82 nM min) ($p < 0.05$). We found no significantly increase in platelet aggregation from baseline till after hormone substitution in the AC-FET group (p-values > 0.16). Likewise, when comparing platelet aggregation with unstimulated controls, no significant changes were found (p-values > 0.51). With data being preliminary, we expect these findings to be more pronounced when investigated in a larger cohort.

Limitations, reasons for caution: We had no wash out period and with a half-life of estrogen on 13-20h, we cannot exclude coagulation parameters to be affected by estrogen before study enrollment. A larger study is required, to confirm our results and to further examine the clinical impact of coagulation changes in women receiving AC-FET.
Wider implications of the findings: Our findings show an increased thrombin generation during estrogen substitution, which might increase the risk of thromboembolism. It is relevant to individually consider the indication for AC-FET and restrict the use of unnecessary hormone exposure.
Trial registration number: 1-10-72-101-19

P-765 Time-related treatment outcomes in infertility studies: a systematic mapping review and evidence-based definition

S.K. Sunkara[1], W. Zheng[2], T. D'Hooghe[2], S. Longobardi[3], J. Boivin[4]

[1]King's College London, Division of Women's Health, London, United Kingdom ;
[2]Merck KGaA, Global Medical Affairs Fertility, Darmstadt, Germany ;
[3]Merck KGaA, Global Clinical Development Fertility, Darmstadt, Germany ;
[4]Cardiff University, School of Psychology, Cardiff, United Kingdom

Study question: How have time-related treatment outcome measures been assessed and reported in clinical studies evaluating infertility treatments and how best to define them?
Summary answer: There is inconsistency amongst studies in reporting time-related treatment outcomes; a robust clinically meaningful contextual definition should be incorporated into all fertility-related clinical studies.
What is known already: Time taken to achieve a healthy live birth is an important consideration when planning fertility treatments. Longer treatment is stressful to patients and increases costs. The ability to compare the effectiveness of interventions with respect to a time-related measure would enable treatment selection aimed at reducing the time to achieve a live birth. The comparison of a time-related measure between possible treatments may also be beneficial for patient counselling with regards to setting expectations. It is, therefore, important to explore how such outcomes have been defined in fertility-related clinical studies with the aim of setting justified and evidence-based definitions.
Study design, size, duration: This is a systematic review of clinical studies in the context of infertility that reported time-related treatment outcomes. PubMed, MEDLINE and Embase were searched for studies published up to 16th August 2019. The search strategy comprised key words/MESH terms: assisted reproductive technologies, fertility treatment, ovarian stimulation, ovulation induction, IVF, intracytoplasmic sperm injection, infertility, subfertility, intrauterine insemination, time, duration, and birth. Results were filtered to include studies in humans and publications in English.
Participants/materials, setting, methods: The screening criteria for inclusion were: a clinical study in the context of infertility (population is infertile or had clinical risk factors for infertility, e.g. fibroids, polycystic ovary syndrome, etc.) and/or a population undergoing fertility management or treatment, which had a treatment outcome measure that contained duration of time (e.g. time to pregnancy or time to live birth). Non-clinical studies, including epidemiological incidence and prevalence studies were excluded.
Main results and the role of chance: 42 out of 5730 captured studies were included in the systematic review after independent assessment for eligibility by two reviewers. The most frequent end time-points for time-related outcome measures were "time to pregnancy" (25/42; 59.5%) and "time to conception" (15/42; 35.7%); 5/42 (11.9%) studies evaluated "time to live birth". There were inconsistencies with regards to the start time-point and no consistent time-related treatment measures reported by majority of studies. We propose time-related treatment measures that build on existing definitions of reproductive outcomes: · Start time-point should be randomization or, for non-randomized studies, the point at which randomization would have occurred if it had been a randomised controlled trial (RCT).

–Kaplan–Meier plot or cumulative incidence plot could be presented to illustrate the likelihood of achieving a clinical pregnancy with a foetal heartbeat that results in a live birth over time (days, weeks, months) or over the number of menstrual or treatment cycles. Medial survival time model could be adapted for example to calculate the time for 25% of the patients to become pregnant (i.e. time to Quartile Pregnancy).

–Data for time-related treatment measures should be reported for all RCTs, regardless of live birth occurrence, and should be adjusted for prognostic covariates.
Limitations, reasons for caution: The review only included studies published in English and authors were not contacted for more details of endpoints evaluated; therefore, some of the time-related treatment measures used in the literature may have been missed.
Wider implications of the findings: Our research provides the basis for a consensus definition of a time-related treatment measure for studies of fertility treatment. We anticipate that our review and justified suggestions will facilitate discussion around appropriate time-related treatment outcome measures for studies evaluating fertility treatments that is relevant to healthcare professionals and patients.
Trial registration number: Not applicable

P-766 Elective transfer of one embryo is associated with higher cumulative live birth rate and improved perinatal outcomes compared to transfer of two embryos with IVF

R. Mejia[1], E. Capper[2], K. Summers[1], P. Ten Eyck[3], B. Van Voorhis[1]

[1]University of Iowa Hospitals and Clinics, Department of Ob-Gyn- Division of REI, Iowa City, U.S.A. ;
[2]University of Iowa Carver College of Medicine, Department of Ob-Gyn, Iowa City, U.S.A. ;
[3]University of Iowa, Department of Biostatistics, Iowa City, U.S.A.

Study question: Are cumulative live birth rate, time to pregnancy and perinatal outcomes similar with initial elective single embryo transfer (eSET) versus double embryo transfer (DET)?
Summary answer: An initial eSET transfer is associated with a higher cumulative live birth rate, a minor increase in time to pregnancy, and markedly improved perinatal outcomes.
What is known already: Compared to DET, eSET is associated with a marked reduction in multiple gestations but has a lower delivery rate per initial embryo transfer. Changes in the Society for Assisted Reproductive Technology (SART) database since 2014 allow linkage of all transfers of both fresh and cryopreserved embryos to their stimulation cycle of origin. This allows for calculation of the cumulative delivery rate per initiated IVF retrieval cycle in a United States national IVF database- a better measure of the total reproductive outcome for patients.
Study design, size, duration: Retrospective cohort study, dataset included a total of 49,333 patients who had their first oocyte retrieval between January 2014 and December 2015. Subsequent frozen transfer cycles linked to the initial retrieval cycle occurring through December 2016 were included for cumulative outcome assessment. The primary outcome was cumulative live birth rate. Secondary outcomes were time to pregnancy resulting in delivery (measured in days and embryo transfer cycles), multifetal pregnancy rate, infant birthweight and perinatal mortality rate.
Participants/materials, setting, methods: Participants included women aged 21 to 45 from a national database of IVF outcomes from SART- reporting fertility clinics. Generalized linear mixed models were used to control for significant clinical and demographic variables when assessing both primary and secondary outcomes between those who received eSET versus DET in the initial embryo transfer.
Main results and the role of chance: As compared to DET, eSET in the initial transfer was associated with a significantly higher cumulative live birth rate (74% vs 57%, AOR 1.32, 95% CI 1.26- 1.38) after controlling for confounding variables. When categorized by the woman's age, a higher cumulative live birth rate with initial eSET was seen in all age categories but only remained statistically significant for women under age 38. In the full sample, initial eSET was associated with a significantly lower cumulative multifetal pregnancy rate (8 % VS 34 %, AOR 0.13, 95% CI 0.12-0.14), a higher birthweight (mean difference of 406 grams, 95% CI 387-425 grams), a lower rate of pre-term births below 28 weeks (1.2% versus 2.8%, p< 0.001) and a lower perinatal mortality rate (0.5% versus 1.2%, p<0.001). Compared to DET, initial eSET was associated with a slightly longer time (82 days versus 51 days, AOR 1.47, 95% CI 1.44- 1.50) and more embryo transfer cycles (1.7 versus 1.4 cycles, AOR 1.19, 95% CI 1.16-1.21) to achieve a pregnancy resulting in delivery.
Limitations, reasons for caution: Categorization of eSET was based on the initial transfer; findings from this study are not generalizable to application of

eSET in subsequent cycles. Despite controlling for many known variables between the eSET and DET groups, other unmeasured differences in patient and cycle characteristics may confound these findings.
Wider implications of the findings: eSET is associated with a higher cumulative delivery rate, especially in younger patients. This combined with the marked reduction in multifetal pregnancies and improved perinatal outcomes suggests that, despite the slightly longer time to pregnancy, a strategy of initial eSET is preferred over DET in the first IVF cycle.
Trial registration number: not applicable

P-767 The prevalence of blood-borne viral infections (BBVI) among roughly 184 00 people seeking fertility services in Ontario, Canada

H. Shapiro[1], M. Neal[2], E. Candido[3], B. Rachlis[3], J. Kwong[4]

[1]University of Toronto, Mount Sinai Fertility, Toronto, Canada ;
[2]Montreal Fertility Centre, Montreal Fertility Centre, Montreal, Canada ;
[3]Institute for Clinical Evaluative Sciences, Primary Care and Health Systems Research Program, Toronto, Canada ;
[4]Institute for Clinical Evaluative Sciences, Populations and Public Health Research Program, Toronto, Canada

Study question: How often is HIV or viral hepatitis diagnosed in first or subsequent testing of people whose testing is solely for the purposes of ART laboratory requirements?
Summary answer: The 5-year cumulative incidence of hepatitis and HIV were 522 (489-556) and 31.4 (23.7-40.6) /100,000.We estimate 80% of HIV cases were identified from initial testing.
What is known already: The incidence and sero-conversion rates of blood-borne viral infections (BBVI) in the fertility-seeking population is though to be very low. Studies to date have not documented any new cases of HIV after initial testing. However, small sample sizes and short duration of follow-up preclude definitive conclusions of incidence or sero-conversion rates. Thus recommendations for testing frequency have been instituted without adequate supportive evidence.
Study design, size, duration: This is an administrative database cohort study of 184 328 individuals who had a consultation for infertility followed by testing for BBVI within one year of that consultation. Data for ongoing testing and consultation for management of BBVI was extracted for up to five years from the first test. The study period was April 1 2002-March 31 2013 for cohort entry and to March 2018 for follow-up.
Participants/materials, setting, methods: The databases used contain coded patient and physician identifiers, service provided, date of service, and diagnosis for Ontario residents in publicly funded healthcare. They do not provide test results. BBVI diagnosis was defined from consultation with a diagnostic code for hepatitis or HIV. This was set as the diagnosis date. The index date was set as the first BBVI test that occurred within one year of the first consultation for infertility.
Main results and the role of chance: 184,328 people had BBVI testing within one year of consultation for infertility. 2491 were found to have pre-existing disease. There were 949 new diagnoses of hepatitis and 57 new diagnoses of HIV in the 5-year follow-up. The cumulative incidence of diagnosis was 522 (95%CI 489-556) per 100,000 for viral hepatitis and 31.4 (95%CI 23.7-40.6) per 100,000 for HIV. The median time from first test to management was 13.0 months (IQR 2.33-32.2) and 2.95 months (IQR 1.34-20.0) for hepatitis and HIV respectively. Thirty nine of the fifty-seven people with HIV were seen in consultation for their BBVI within one year of the first test. The interval from first test to management for women with HIV was much shorter than for men. Forty-four percent of patients had one BBVI test prior to management. The majority who had two tests before BBVI consultation had the second test less than one year from the first. The median time from last test to management was 2.39 (<1-9.28) and 1.51 (<1-5.41) months. Sixty-two individuals had five or more BBVI tests prior to a diagnosis of hepatitis. The incidence of HIV after multiple BBVI tests was too small to be reliably calculated.
Limitations, reasons for caution: Every positive test may not have resulted in consultation, underestimating the incidence of BBVIs. Testing for clinical indications could not be separated from testing as ART routine practices, which would overestimate the incidence. Consultation more than one year from initial testing may reflect either delay to care or interim sero-conversion.
Wider implications of the findings: *This is the largest population-based study describing BBVI testing and disease incidence in the infertility population. The* majority (but not all) of HIV diagnoses were made with the first test. These results can be used to develop evidence-based, cost-effective guidelines for BBVI test frequency in ART.
Trial registration number: not applicable

P-768 Endometrial thickness before embryo transfer is associated with placenta accreta at delivery.

O. Rosenbluh[1], A. Peretz[1], D. Lasry[2], A. Solnica[3], E. Esh-Broder[1], D. Hochner-Celnikier[1]

[1]Hadassah Medical Center, Obstetrics & Gynecology, Jerusalem, Israel ;
[2]Shaare Zedek Medical Center, Obstetrics & Gynecology-, Jerusalem, Israel ;
[3]Hadassah Hebrew University, Henrietta Szold School of Nursing, Jerusalem, Israel

Study question: Our objective was to explore the correlation between endometrial thickness before embryo transfer and the finding of placenta accreta at delivery among patients utilizing in-vitro fertilization (IVF) treatments.
Summary answer: Thinner endometrial thickness before embryo transfer is an independent risk factor for developing placenta accreta during pregnancy among IVF patients.
What is known already: It has been shown that besides prior cesarean section, placenta previa and advanced maternal age, in-vitro fertilization (IVF) is an independent risk factor for placenta accreta.

Recently, it has been suggested that a correlation exists between the endometrial thickness at time of embryonic transfer and the risk of placenta accreta, claiming that the thinner the endometrium is at time of transfer the more prominent the finding of placenta accreta at birth.
Study design, size, duration: This is a case-control study where we retrospectively analyzed the medical records of 29 cases of placenta accreta occurring in IVF patients, who were treated and gave birth at our Medical Center between the years 2007-2017. We then created a control group of IVF patients matched by age, date of embryo transfer and prior cesarean section (1:2) without accreta and compared the endometrial thickness before transfer between the two groups.
Participants/materials, setting, methods: All patients, who delivered a live-born fetus at 24 weeks of gestation or greater at our Center from 2007 to 2017, were reviewed for placenta accreta. Of those, we designated a subset of women undergoing fertility treatments who were both clinically adherent and showed invasive pathology (n=29).

Cases of accreta were matched to controls (1:2) without accreta, and multivariable logistic regression model was performed, while controlling for potential confounders.
Main results and the role of chance: Among patients with placenta accreta who were both clinically adherent and reported as having invasive pathology, higher rates of thinner (under 7 mm) endometrial linings were observed as compared to patients who did not develop placenta accreta (21.4% vs. 5.2% respectively, p<0.05). While performing sub-analysis we observed that lower endometrial thickness was significant only when comparing the transfer of fresh embryos (33.3% vs. 5.0%, p<0.05). However, when comparing cryopreserved embryo transfer no significant association was observed (7.7% vs 5.5%, p=0.81). In multivariate analysis, lower endometrial thickness is an independent risk factor for placenta accreta while controlling for maternal age, prior cesarean section and fresh vs. frozen (odds ratio 4.07, 95% confidence interval 1.1-16.5)
Limitations, reasons for caution: The limitation of the study is the retrospective case-control nature of the study.
Wider implications of the findings: We have determined that patients who developed accreta had thinner endometrial linings compared to patients without accreta in fresh embryo transfers. A threshold value of 7 mm or less was shown to be significantly higher in accreta cases requiring special attention be paid to endometrial thickness during fresh IVF cycles.
Trial registration number: N\A

P-769 Maternal outcomes of IVF twins versus spontaneous twins

T. Nguyen[1], D. Tran[2], T. Phan[3], D. Do[4], M. Nguyen[5], H. Nguyen[5], A. Salumets[6]

[1]Ha Noi Obstetric and Gynecology Hospital, Department of High risk Pregnancy, Ha Noi, Vietnam ;

[2]Ha Noi Obstetrics and Gynecology Hospital, Department of High risk Pregnancy, Ha Noi, Vietnam ;
[3]Ha Noi Obstetrics and Gynecology Hospital, Delivery Department, Ha Noi, Vietnam ;
[4]Ha Noi Obstetrics and Gynecology Hospital, Gynecology Department, Ha Noi, Vietnam ;
[5]Ha Noi Obstetrics and Gynecology Hospital, Assisted Reproductive Center, Ha Noi, Vietnam ;
[6]University of Tartu, Women's Clinic and Institute of Genomics, Tartu, Estonia

Study question: Investigate the potential differences in maternal outcomes between IVF and spontaneous twins in the context of a low-middle income country
Summary answer: Our study shows that although there are some differences in perinatal complications, pregnancy overall outcomes were not significantly different between IVF and spontaneous twins.
What is known already: Assisted reproductive technologies have been used widely in obstetrics, especially invitro fertilization (IVF). One of the main concerns of both patients as well as doctors is how IVF techniques affect the maternal outcomes of the pregnancy. Many studies were conducted to address this question but few have been done in the context of a low-income country like Vietnam where multiple embryo transfer remains popular and the demand of multiple pregnancies is still high.
Study design, size, duration: Size: 733 women with twin pregnancy were included in the study (344 IVF twins and 389 spontaneous twins).
Duration: 6 months from 1 Jan 2019 to 30 June 2019
Participants/materials, setting, methods: A retrospective cohort study was performed on women with twin pregnancy delivered at a large public women's hospital in the North of Vietnam in 6 months from 1 Jan 2019 to 30 June 2019. Two groups of twin pregnancies were investigated: IVF and spontaneous ones. Anonymous maternal outcome information was extracted from the hospital database.
Main results and the role of chance: 733 women with twin pregnancy were included in the study (344 IVF twins and 389 spontaneous twins). When it comes to maternal prenatal complications, higher rates of first-trimester bleeding (5.8% vs 1.8%, p<0.01), gestational diabetes (12.79% vs 7.2%, p<0.01) and pre-eclampsia (8.72% vs 2.83%, p<0.001) were seen in IVF group, compared to control group, respectively. No HELLP syndrome, abruptio placentae or placenta accreta cases were reported. Despite the percentage of threaten preterm birth was higher (38.7% vs 14.4%, p<0.001, respectively) and the gestational ages at delivery were lower in the IVF group (36.2 ± 2.5 weeks vs 36.5 ± 2.4 weeks, p<0.05), the actual rates of preterm birth and birthweight were not different between the two groups (65.7% vs 66.3%, p>0.05 and 2372.8 ± 467.0 grams vs 2323.3 ± 493.1 grams, respectively). IVF women tended to have more C-section than those who conceived twins naturally (84.01% vs 74.29%, p<0.001, respectively). The proportions of postpartum uterine atony and bleeding were higher in IVF group (20.6% vs 14.4%, p<0.05 and 28.2% vs 17.7%, p<0.01, respectively) but those of blood transfusion or perinatal infection were similar between the two groups.
Limitations, reasons for caution: Our study is a retrospective cohort study
Wider implications of the findings: With careful monitor and follow-up, despite higher risk of antenatal complication, IVF twin pregnancies may achieve similar outcomes as spontaneous twins.
Trial registration number: not applicable

P-770 Long-term follow-up of children conceived with assisted reproductive techniques – attention-deficit hyperactivity disorder and 9th-grade performance in the Swedish population.

C. Wang[1], A.L.V. Johansson[1], C. Almqvist Malmros[1,2], S. Hernández-Díaz[3], A.S. Öberg[1,3]

[1]Karolinska Institutet, Medical epidemiology and Biostatistics, Stockholm, Sweden ;
[2]Karolinska University Hospital, Astrid Lindgren Children's Hospital, Stockholm, Sweden ;
[3]Harvard University, Harvard T.H. Chan School of Public Health, Boston, U.S.A.

Study question: Could use of assisted reproductive techniques (ART) affect offspring risk of attention-deficit hyperactivity disorder (ADHD) or 9th grade school performance?
Summary answer: We found no evidence of a negative influence of ART on children's risk of ADHD or their academic achievement in lower secondary school.
What is known already: Early neurodevelopmental assessments of children conceived with in-vitro fertilization (IVF) have been largely reassuring, though a reported link between use of intra-cytoplasmic sperm injection (ICSI) and risk of autism warrants further attention. Some deficiencies may not become manifest or of significance until school-age (ADHD) or adolescence (academic achievement), and larger studies with longer follow-up are needed to understand the long-term consequences of IVF (and ICSI), independent of infertility.
Study design, size, duration: Linkage of national registers allowed prospective follow-up of all live births in Sweden up to the end of 2012. To allow the outcomes to be captured, school performance was evaluated in the cohort born 1983 to 1996 ((N=1,493,903) whereas ADHD was evaluated in the cohort born 1997 to 2006 (N=926,198).
Participants/materials, setting, methods: Use of ART (IVF/ICSI) was reported by IVF-clinics, medical records, and mothers at the first antenatal visit. Cases of ADHD were identified via specialist diagnosis (outpatient register) and/or use of ADHD medication (prescribed drug register). The school register provided total grade score and eligibility for upper secondary school. Children conceived with ART were compared to spontaneously conceived children (1) from the population, and (2) from couples with known trouble conceiving, while adjusting for background factors.
Main results and the role of chance: The study included a total of 2,420,101 children. Overall, children conceived with ART were at lower risk of ADHD (Hazard Ratio (HR)=0.81 [95% CI 0.75-0.88]) and did slightly better in lower secondary school (mean difference in the grade average (scale 0-20) (MD)=1,10 [95% CI 1.01-1.20]; eligibility Odds Ratio (OR)=1.45 [95% CI 1.31-1.61]), but once maternal characteristics were taken into account they rather appeared at a slight disadvantage (with no difference in risk of ADHD). In the comparison to children of couples with known trouble conceiving, a similar overall advantage was simply attenuated toward the null (HR=0.94 [95% CI 0.86-1.02]; MD=0.05 [95% CI -0.04-0.25]; OR=1.01 [95% CI 0.90-1.10]). Further comparison of children conceived with IVF alone versus with IVF and ICSI showed no differences in risk of ADHD.
Limitations, reasons for caution: The study concerns all children born in Sweden across two decades. Variation in clinical practice between countries is expected but may concern access to and indications for IVF/ICSI more than the medical procedures themselves. Treatment strategies have also changed over time, with single-embryo transfer policy e.g., reducing multiple gestations.
Wider implications of the findings: This study provides additional reassurance concerning offspring neurodevelopment following ART, finding no indication for concern about children's risk of ADHD or overall performance in school.
Trial registration number: not applicable

P-771 Artificial intelligence (AI) is an emerging topic in the fertility field: it is time to discuss standards of reporting AI outcomes

J. CHAMBOST[1], C. Jacques[1], C.A. Pena[1], M. Kotrotsou[2], C. Hickman[3]

[1]Apricity, AI team, Paris, France ;
[2]Apricity, Medical team, London, United Kingdom ;
[3]Apricity, AI team, London, United Kingdom

Study question: How has the reporting of AI evolved in the last two years at the annual conferences of ASRM, ESHRE and Fertility?
Summary answer: Volume of AI studies in fertility is increasing, but the quantity and quality of data remains low compared to AI in other healthcare fields
What is known already: AI has become a mainstream topic in various healthcare industries, including oncology, radiology and genetics. In fertility, Curchoe and Bormann (2019) demonstrated that AI publications at ESHRE (the largest worldwide conference in fertility, based in Europe) and ASRM (the second largest worldwide conference in fertility, based in America) increased sharply from 2017 to 2018. At ESHRE, from one to seven, and at ASRM, from one to nine. The

authors observed wide variability in how AI methods were described as well as size of datasets (from 3 to 11,898) and concluded that there was a need for applying reporting standards.
Study design, size, duration: Systematic review of conferences ASRM 2018 vs 2019, ESHRE 2018 vs 2019 and Fertility (largest UK conference in fertility) 2019 vs 2020. Program, abstract books and conference applications, as well as attendance to all six conferences, were used to collect information on all relevant abstracts, whether presented as poster or oral.
Participants/materials, setting, methods: 4986 abstracts were analysed: 1064 for ASRM 2018, 1089 for ASRM 2019, 1104 for ESHRE 2018, 1116 for ESHRE 2019, 293 for Fertility 2019 and 320 for Fertility 2020. Using PRISMA method, the abstracts were searched using the following keywords: artificial intelligence, deep learning, machine learning.
Main results and the role of chance: All three conferences reported an increase in AI publications over time: an overall increase of 1.7 fold from one year to the next (19 to 32). ASRM increased by 1.8 fold (from 9 in 2018 to 16 in 2019), ESHRE increased by 1.4 fold (from 7 in 2018 to 10 in 2019), Fertility increased by two fold (from 3 in 2019 to 6 in 2020). AI still represents a minor proportion (1%) of all abstracts. Many areas of assisted reproduction were represented, with embryo quality assessment, ploidy or viability prediction, sperm motility or quality assessment, IVF stimulation protocols and recipient-donor matching. Most studies were focused on deep learning analysis on images (81%) to improve embryo selection (69%). As described in Curchoe and Bormann (2019), high variability in data sample size continues to be the case (from 50 to 20,000 images for embryo selection topics using deep learning) with most studies analyzing a few thousands of images, which is insufficient for deep learning standards. Variability was also observed in algorithm prediction results (from 50% to 82% for classification accuracy) and all abstracts lacked sufficient details on the algorithm structure to allow for suitable scrutiny as to the validity of the claims.
Limitations, reasons for caution: This study assessed conference abstracts and associated presentations rather than publications. Of all AI abstracts identified in Fertility 2020, two were published, neither of which reached the recommended standards for AI publication seen in other fields. Studies and datasets could overlap, reducing the actual number of independent studies on AI
Wider implications of the findings: Results further emphasize the importance of setting standards for AI studies reporting and comparison. The relatively small sample sizes suggest a need for a secure data sharing infrastructure that would enable effective and high quality collaborative AI studies on sensitive data that will ultimately benefit patients.
Trial registration number: not applicable

P-772 Human error measurement and human error reduction with electronic witnessing system (EWS)

B. Aydin[1]

[1]*Ivmed Clinic Fertility Center, Embryology, Kiev, Ukraine*

Study question: Is human error during routine IVF process measurable? At what stages can human error occur most, and how can it be minimized?
Summary answer: Human error in routine IVF can be measured with EWS. With our RFID chip EWS, human error can be reduced to very low levels.
What is known already: Existing studies show that electronic witnessing system works very successfully in all stages of IVF applications, but it is still insufficient in terms of cryopreservation and software. EWSs should secure all stages of IVF process from the time patient enters the clinic to the completion of all laboratory procedures. Our study is designed to ensure biological material safety with EWS and is intended to reveal statistical data of a system that can be actively used in all laboratory processes. We also determined the cryopreservation process and the performance of all IVF personnel with the system.
Study design, size, duration: 15000 IVF cycles covering the years 2016-2020 were recorded with IVFID Electronic Witnessing system. Error warning received from 36 different IVF clinics were calculated and error distributions at each IVF stage were determined. Normal IVF patients, patient group for egg donation and surrogacy were registered, and IVF stages affected by human error were determined by calculating records of possible errors for each patient group.
Participants/materials, setting, methods: RFID electronic chip, electronic wristband and barcode system were used in every stage of patient groups. Thus, biological materials were secured throughout the entire laboratory process. The system recorded the data received via electronic chip through software and calculated it statistically. Human errors from each procedure in the embryology and andrology laboratories were recorded. In addition, the system was supported with personal witnessing patient software, and the error rate was reduced to zero.
Main results and the role of chance: In our study where 15,000 IVF cycles were evaluated, human error data received from 36 different clinics were evaluated and statistically calculated. Human error warnings were received 169 times out of 15,000 IVF cycles at different stages. Accordingly, error warnings were received 14 times during the oocyte pick up phase, 17 times during the denudation phase, 26 times during the ICSI phase, 8 times during the dish change phase, 68 times during the embryo transfer phase (17 of these were received during the fresh embryo transfer phase and 51 times during the frozen embryo transfer phase) and 36 times during the sperm preparation phase. When the error distribution according to different clinics were evaluated, error warnings were received from 23 out of 36 different clinics at different stages. Human errors were prevented by RFID electronic chip system and embryologist was warned visually and audibly on screens during the procedure. During the cryo phase of the IVFID Witnessing system with the vitrification straw chip system, no error warnings were received. Looking at individual embryologist performances, it is seen that error warnings were received from 32 different embryologists.
Limitations, reasons for caution: EWS's purpose is reducing human error and ensuring biological materials' safety. The use of system is important at every stage in Embryology/Andrology Laboratories. System can only send alerts regarding certain human errors, so 100% biological material safety isn't guaranteed. Human factor will always exist, and individual witnesses should support EWS.
Wider implications of the findings: Regular use of EWSs in IVF laboratories is very important to avoid human error-based interferences in biological materials, and they should be used regularly in IVF laboratories. In addition, EWSs can be actively used in genetic laboratories during the IVF process and clinical laboratories.
Trial registration number: not applicable

P-773 A new tool to evaluate the performance of IVF Centres

P. Arvis[1], **M. Benchaib**[2]

[1]*Clinique La Sagesse, ART, RENNES, France ;*
[2]*Hôpital Femme Mère Enfant, Laboratoire Biologie de la Reproduction, Lyon, France*

Study question: Our objective was to develop an indicator to evaluate the performance in ART, based on a very large database, and using artificial intelligence.
Summary answer: The segmentation of the treated population into 4 different prognostic classes allows a much more precise evaluation of the actual performance than the overall result.
What is known already: The overall performance of IVF Centers ,, based on Cumulative Live Birth Rate (CLBR), depends on multiple factors, including setting criteria for measuring the success, initial patient selection, practitioner competence, choice of protocols, quality of laboratory and equipment, and completeness of data collected. As a result, it is very difficult to compare performance among IVF Centers on objective and homogeneous criteria. However, the main question is to determine up to what extent the success rate depends on the skill of practitioners and laboratories, or on the initial selection of good prognosis patients. A tool is needed to answer this question.
Study design, size, duration: This non-interventional retrospective controlled study included a total of 113,253 unselected consecutive cycles (81.268 Controlled Ovarian Stimulation [COS], and 31985 Frozen embryo transfer [FET]), collected from 12 IVF French centres during 2007–2016 period. No exclusion criteria were applied, excepted for oocyte donation or preservation. This database contains complete and detailed information on patients' history and characteristics, protocols used, ovulation monitoring, oocyte collection, embryo production, transfer and freezing, and pregnancy outcome.
Participants/materials, setting, methods: The primary endpoint was the CLBR. The model was developed using the machine learning on a training set

(n=56993). Simple and multiple logistic regressions were used. From the odds ratios a selection indicator was calculated with a geometric mean, this value was divided into 4 classes. The best prognosis modality, of each selected covariates has been chosen as reference, the geometric mean cannot exceed 1. The results obtained were validated on a setting set (n=24426).

Main results and the role of chance: The multivariate analysis on the training set shows 7 baseline covariates linked to CLBR, both highly significant and independent: female age, BMI, AMH level, uterine abnormality, rank of attempt, previous abortion, parity. The combination of these indicators and their influence on the CLBR allows a selection indicator to be calculated, reflecting the overall quality of the initial selection of patients for each Centre.

We defined 4 prognosis levels for this selection indicator, with decreasing probability of CLBR (A=29,6% [22,1-36,8] - B =21,3% [18,0-30,1] - C=13,4% [6,4-20,5] - D=8,1 [3,0-14,2]), and marked differences between Centres.

The range of distribution of the 4 levels highlights the differences in initial selection of patients, especially in extreme values (A=17,2% [6,5-25,0] – B= 48,9% [43,8-52,8] – C=17,3% [11,3-19,9] – D=16,6% [9,8-26,2]).

Centres were ranked in descending order 1 to 12, according to their global CLBR. We calculated what the CLBR of each Center would have been in a standard population, by applying their own success rate in each prognosis class A-B-D-D at the standard distribution of the prognosis classes. Under this approach, the final rank order attributed to all Centers based on their global success rate is completely different from the initial rank order, namely: 1-12-7-8-2-11-9-5-6-3-4-10.

Limitations, reasons for caution: Potential biases might still exist due to missing values, in spite of statistical adjustment.

Wider implications of the findings: This calculation method of the success rate allows to disconnect the role of the initial selection of patient from the expertise of Centres. The segmentation of the treated population into 4 different prognostic classes allows to identify the Centres with specific expertise in the management of the most difficult cases.

Trial registration number: not applicable

P-774 Assessment of operator performance during oocyte retrievals: Residents' learning curve and continuous monitoring of senior physicians.

C. Verhaeghe[1], L. Inchboard[2], R. Corroenne[1], C. Dreux[1], P. Jeanneteau[1], H. El Hachem[3], P. May-Panloup[1], P.E. Bouet[1]

[1]Angers University Hospital, Department of Reproductive Medicine, Angers, France ; [2]Angers University Hospital, Clinical Research Center, Angers, France ; [3]Clemenceau Medical Center, Department of Reproductive Medicine, Beirut, Lebanon

Study question: How does the performance of residents and senior physicians during oocyte retrievals (OR) evolve over time?

Summary answer: A resident needs a mean number of 62 retrievals to reach clinical proficiency, while senior physicians maintain an adequate performance level over time.

What is known already: Only one study looked at the OR learning curve of residents, and reported a large variability between students. Indeed, it found that anywhere between 17 and 50 retrievals were needed to obtain an acceptable performance level. The learning curve cumulative summation test (LC CUSSUM test) allows to define an individualized learning curve and determine the moment when clinical proficiency is attained. After acquisition of the skills, the cumulative summation test (CUSSUM test) allows to monitor the maintenance of the required level over time.

Study design, size, duration: We performed a retrospective study at Angers university hospital between May 2017 and September 2018. 7 Obstetrics and Gynecology residents and 5 senior physicians were included, and all OR performed during that time (n=690) were analyzed.

Participants/materials, setting, methods: During OR, the operator counted the number of follicles punctured in each aspiration tube (At our center, all follicles ≥ 8 mm are punctured during OR). Each tube had a technical sheet containing the name of the operator and the number of follicles. The performance index assessed was the oocyte retrieval rate (ORR), defined as the ratio of oocytes retrieved to follicles punctured.

Main results and the role of chance: 690 OR were included: 315 were performed by residents, 220 by senior physicians, and 139 by both residents and physicians (mixed retrievals). We defined an ORR ≥50% in 60% of retrievals as the threshold of clinical proficiency. 4 residents (57%) (residents 1, 2, 3, and 4) reached the threshold after puncturing 82 67, 53 and 46 ovaries, respectively. The mean number of ovaries punctured in order to reach clinical proficiency was 62, and the mean number of weeks needed was 21. The three remaining residents did not reach the threshold during the observation period: two of them had only been training in the department for 2 months, and the third had a partial training schedule (50%). All the other residents had been training in the department for 6 months. Two senior physicians (40%) remained proficient across the duration of the study, while two physicians (40%) had one statistically "suboptimal" OR, and one physician (20%) had two suboptimal retrievals.

Limitations, reasons for caution: The main limitation of our study was the lack of randomization of the ovaries to be punctured between residents and physicians in mixed retrievals. However, we believe it would be difficult to randomize such cases with the consent of the couples.

Wider implications of the findings: The number of OR needed to achieve clinical proficiency varies between residents. Our study shows that a mean number of 62 retrievals and a full training schedule of 6 months in the department is required in order to reach the acceptable level.

Trial registration number: NA

P-775 Chromosomal abnormalities in males, but not chromosomal polymorphisms, affect the preterm delivery rate in ICSI+PGT cycles

F. Rodriguez Valderrama[1], M. Cruz Palomino[2], J. Pla Victori[3], A. Requena Miranda[2]

[1]Universidad Rey Juan Carlos, PhD student of Health Science, Madrid, Spain ; [2]Instituto Valenciano de Infertilidad, IVIRMA Global, Madrid, Spain ; [3]Instituto Valenciano de Infertilidad, IVIRMA Global, Barcelona, Spain

Study question: Do Chromosomal abnormalities (CA) or chromosomal polymorphisms (CP) in males affect the incidence of congenital alterations or pregnancy outcomes?

Summary answer: CA or CP in males don't affect the congenital alteration rate in ICSI+PGT babies, but preterm delivery rate is higher when CA are present

What is known already: IVF couples often present male/female idiopathic infertility, which could have a genetic origin. CA are known to be a major cause of gametogenesis failure. For these couples, PGT is often offered, which reduces the chance of miscarriage, birth defects, and the probability of live newborns with unbalanced CA.

CP are known as benign variants of chromosomes. Recently, some reports have shown conflicting results on their effect on IVF outcomes. Some studies found no effect, whereas others concluded that CP were associated with adverse effects, such as lower fertilization rate, implantation rate, cleavage rate, clinical pregnancy rate and live birth rate.

Study design, size, duration: A multi-center retrospective cohort analysis of autologous IVF cycles with embryo transfer performed between January 2015 and December 2018. All couples underwent karyotype screening before IVF treatment, and all females had normal karyotypes. Only cycles where euploid embryos were transferred and had at least one live newborn baby were included. Primary outcomes measured were congenital alterations rate and preterm delivery rate.

Participants/materials, setting, methods: Couples were divided in three categories according to the male karyotype: group A males with normal karyotype, Group B males with chromosomal polymorphisms and group C males with abnormal karyotype. Sperm concentration, sperm motility, fertilization rate, abnormal embryo rate, frequency of congenital alterations, low birth weight and preterm delivered were compared between the groups. Data obtained was assessed in terms of clinical outcome and statistically analyzed with ANOVA test and Chi-squared test.

Main results and the role of chance: In total 1798 couples were included in the analysis: 1698 in group A, 44 in group B and 56 in group C. There were significant differences in sperm concentration and non-progressive sperm between Group A and C but not with Group B (41.1 ± 1.1 (A) vs. 34.7 ± 10.0 (B) vs. 26.6 ± 9.6 (C), p=0.004 and 9.6 ± 0.3 (A) vs. 10.4 ± 1.7 (B) vs. 14.4 ± 6.2 (C), p<0.001,respectively).

Fertilization rate and abnormal embryo rate differed significantly between groups (FR: 76.8% (A) vs. 71.8% (B) vs. 68.8 (C), p=0.001 and AER: 46.1% (A) vs. 50.0% (B) vs. 54.8% (C), p=0.043), with group A showing the best results, followed by group B. In the case of pregnancy outcomes, group A showed better results in preterm delivery compared with group C, but not with group B (7.5% (A) vs. 5.3% (B) vs. 15.6% (C), p=0.045). Low birth weight didn't show significant difference between groups, neither did congenital alterations rate (LBWR: 8.0% (A) vs. 8.0% (B) vs. 3.3% (C), p=0.860 and CM: 4.4% (A) vs. 2.2% (B) vs. 5.3% (C), p=0.087).

Limitations, reasons for caution: The main limitation is the retrospective nature of the study, although known and suspected confounders were adjusted. Also, couples with CA are more prone to early pregnancy check-ups in order to diagnose congenital alterations and therefore perform pregnancy interruptions, the frequency of congenital alterations might be higher in this group.

Wider implications of the findings: Our results show that the incidence of congenital alterations is not affected by the presence of CA or CP in the male partner of couples undergoing ICSI+PGT cycles, but the risk of preterm delivery is slightly higher when CA are present.

Trial registration number: N/A

P-776 Obstetric and perinatal outcomes in IVF cycles in which transdermal testosterone has been used during ovarian stimulation

R. Solernou[1], F. Fabregues[1], J. Ferreri[1], E. Vidal[1], M. Solsona[1], S. Peralta[1], A. Goday[1], G. Casals[1], A. Borras[1], D. Manau[1]

[1]Hospital Clínic of Barcelona, Assisted reproduction department, Barcelona, Spain

Study question: Is transdermal testosterone (TT) supplementation in IVF cycles safe in terms of obstetric and perinatal outcomes?

Summary answer: The administration of transdermal testosterone in poor responders (PR) undergoing IVF treatment does not convey a higher risk of obstetric and perinatal complications

What is known already: Many strategies have been tried to improve the results in poor responder patients. Androgen supplementation with transdermal testosterone is the only one that has significantly increased live birth rate (LBR) in these patients. However, the offspring safety has not been analyzed

Study design, size, duration: This is a retrospective cohort study of 126 newborns coming from singleton pregnancies of poor responder patients according Bologna criteria performed between 2015 and 2018

Participants/materials, setting, methods: 126 newborns from singleton pregnancies of PR patients according to Bologna criteria were included within 2015-2018. Study group included 46 newborns from pregnancies in which TT supplementation was administered and a control group of 80 newborns in which TT was not administered. We analyzed birth weight, gestational age at delivery, incidence of preeclampsia, intrauterine growth restriction, gestational diabetes, congenital malformations and genetic disorders. In the study group TT was administered 5 days preceding ovarian stimulation

Main results and the role of chance: There were no differences in both groups in maternal age, BMI, smoke condition or duration of infertility. No significant differences were found between both groups (TT vs no supplementation) in obstetric and perinatal outcomes: birth weight (3210±421g vs 3190±426g; p=0.85), gestational age at delivery (39.4weeks vs 39.2 weeks; p=0.64), incidence of preeclampsia (2.1% vs 6.2%; p=0.36), intrauterine growth restriction (9.5% vs 5.1%; p=0.17), gestational diabetes (17.4% vs; 11.3% p=0.49), congenital malformations and genetic disorders (2.1% vs 2.4% p=0.87)

Limitations, reasons for caution: This a retrospective study with limited number of patients included. Studies are required to evaluate long-term health parameters

Wider implications of the findings: This is the first study to asses obstetric and perinatal outcomes of newborns from an IVF cycle with TT supplementation. Although randomized clinical trials are required, there seems to be no harm to the newborns health.

Trial registration number: not applicable

P-777 Dynamic view of assisted reproduction in Turkey from 1996–2018: Trends and changes in patient characteristics, clinical and laboratory practice

K. Yakin[1], B. Balaban[2], B. Urman[1]

[1]Koc University School of Medicine, Obstetrics and Gynaecology Department, Istanbul, Turkey ;
[2]American Hospital, Assisted Reproduction Unit, Istanbul, Turkey

Study question: What are the trends in patient demographics and practices in assisted reproduction treatment (ART) over the last two decades?

Summary answer: There are substantial changes in female age, indications, ovarian stimulation protocols, ovarian response, utilization of blastocyst culture and single embryo transfer, and multiple pregnancy rates.

What is known already: The last two decades have witnessed considerable changes in patient demographics and improvements in laboratory conditions, culture media and cryopreservation techniques.

Study design, size, duration: A retrospective cohort study of 24819 women who underwent autologous ART treatment in a single private center between 1996 and 2018.

Participants/materials, setting, methods: Data regarding female age, duration of infertility, treatment indication, number of previously failed treatment cycles, ovarian stimulation protocols, number of oocytes retrieved, stage of embryo development at the day of transfer, number of embryos transferred, fresh or frozen-thawed embryo transfer, method for cryopreservation, implantation, pregnancy, and multiple pregnancy rates were extracted by the calendar year of treatment. Interrupted time series and linear regression analyses were used to assess changes in patient characteristics, trends and outcomes.

Main results and the role of chance: Over 23 years during which the data was collected, there was a steady increase in the average age of women undergoing ART from 32.0 to 37.4 years. The duration of infertility showed a downward and the average number of previously failed cycles showed an upward trend. The major indication for ART was male factor infertility until 2001, and then it was gradually replaced by diminished ovarian reserve and unexplained infertility. GnRH antagonist protocols gradually took over GnRH agonist protocols. The mean number of retrieved oocytes decreased from 11.5 to 6.9. The mean number of embryos transferred decreased from 3.9 to 2.4 until 2010, and then to 1.5 following a legislative restriction. Blastocyst stage transfers were started in 1998 and were gradually increased to 50.8% of all transfers by 2018. While multiple pregnancy rates decreased from 31% to 9%, clinical pregnancy rates per embryo transfer were constant between 1997-2018, ranging between 31.6-43.9%. Clinical pregnancy rates in frozen-thawed embryo transfers showed a significant increase after 2015, in parallel with increased utilization of blastocyst vitrification.

Limitations, reasons for caution: This is a retrospective cohort analysis from a single center and does not intend to confirm any causality.

Wider implications of the findings: ART practice in Turkey has evolved in parallel with worldwide trends. While IVF patients are getting older, increased utilization of single embryo transfer at the blastocyst stage and improvements in cryopreservation techniques helped maintain high live birth rates and reduced multiple births.

Trial registration number: Not applicable

P-778 Impact of cell loss after warming of human vitrified day 3 embryos on the obstetrical outcome in single frozen embryo transfers

A. Racca[1], G. Coticchio[2], F. Di Guardo[3], A. Borini[2], P. Drakopoulos[3], S. Mackens[3], H. Tournaye[3], G. Verheyen[3], C. Blockeel[3], L. Van Landuyt, Belgio[3]

[1]UZ Brussel, CRG - Centre Reproductive Medicine, Brussels, Belgium ;
[2]9.baby, Reproductive medicine, Bologna, Italy ;
[3]UZ Brussel, CRG - Centre Reproductive Medicine, Brussel, Belgium

Study question: Does cell loss (CL) after vitrification and warming (V/W) of day 3 embryos have an impact on live birth rate (LBR) and neonatal outcomes?

Summary answer: LBR is significantly higher with intact embryo as compared with an embryo with CL after V/W. Neonatal outcomes are comparable between the two groups.

What is known already: The use of frozen embryo transfer has progressively increased in the last two decades due to the advances in the development of efficiency and safety of cryopreservation techniques, especially in

vitrification. Indeed, vitrification has replaced the slow-freezing technique as an effect of its improved survival rate, fully intact embryo rate and implantation rate as compared with slow-freezing procedures. However, day 3 vitrified embryos with CL after warming, showed lower overnight cleavage rate than fully intact embryos in both methods. In this context, it is interesting to investigate whether the CL may impact pregnancy rate, LBR and neonatal outcomes.

Study design, size, duration: This present retrospective analysis includes all cleavage stage day 3 embryos (n=2334) that were vitrified and warmed between 2011 and 2018. Only single vitrified/warmed embryo transfers were included. Each woman was included only once in the analysis. Pre-implantation genetic screening, oocytes donation and age banking were excluded from the analysis.

Participants/materials, setting, methods: The sample was divided into two groups, namely group A (intact embryo after warming) and group B (<50% blastomere loss after warming). The vitrification method used was closed vitrification using CBS-VIT High Security straws with D

Main results and the role of chance: A total of 2334 embryos were included in the analysis. Group A contained 1954 fully-intact embryos (83.7%), while group B had 380 (16.3%) CL embryos after V/W. In group B, the majority of the embryos had lost one cell (235 embryos, 61.8% of the CL-embryos). Demographic characteristics such as age at cryopreservation, cause of infertility, insemination procedure and semen origin were comparable between the two groups. The positive hCG rate (30% and 23.9%, respectively for intact vs CL group, p=0.018) and LBR (13.7% and 11.6%, respectively for intact vs CL group, p=0.018) per warming cycle were significantly higher in the intact embryos group; however, the LBR per positive hCG was equivalent between intact and damaged embryos (45.6% vs 38.5%, respectively, p=0.2). Newborn measurements (length, weight and head circumference at birth) showed no statistical difference between the two groups. Multivariate logistic regression was performed in order to adjust for potential confounders and it showed that intact vs CL embryos is not predictive of LBR when holding for patients' age and embryo quality.

Limitations, reasons for caution: The major limitation of the present study is its retrospective design. However, this study question would not benefit from a prospective design as it wouldn't be possible to control for embryo damage and deciding to transfer intact or a no-intact embryo would not reflect the real clinical setting.

Wider implications of the findings: This is the first study providing evidence that CL due to V/W of cleavage stage embryos doesn't influence newborn measurements. Vitrification of cleavage stage embryos is associated with lower pregnancy and live birth rates when there is blastomere loss. However, once implantation ensues, LBR is not hindered by cell loss.

Trial registration number: not applicable

P-779 Conception by means of in vitro fertilization (IVF) is not associated with nausea and vomiting of pregnancy

F. Bazargani[1], A. Skalkidou[1], E. Elenis[1]

[1]Uppsala University, Department of Women's Health- Uppsala University Hospital- Uppsala- Sweden, Uppsala, Sweden

Study question: Is there any association between mode of conception or IVF-related variables and nausea and vomiting of pregnancy (NVP)?

Summary answer: Conception by means of IVF is not associated with NVP but the stage of the transferred embryo may affect NVP development.

What is known already: The exact cause of NVP is unknown but risk factors including increased hormonal levels, maternal distress and anxiety disorders, also described in IVF populations, have been reported. There are only a few studies exploring NVP in IVF samples. A population-based study examining the characteristics of women who suffered from a severe form of NVP, it was reported that women with severe NVP had more often conceived through assisted reproduction techniques. So far, the relationship between NVP and IVF or different treatment related parameters in the IVF population in relation to NVP remains unclear.

Study design, size, duration: The study is a longitudinal, matched - cohort, pilot study including 630 pregnant women with singletons without malformations, recruited during the pregnancy ultrasound in gestational week 17 (GW 17). The study was conducted between 2010-2016 at the University Hospital of Uppsala, Sweden.

Participants/materials, setting, methods: The study population comprised 210 women with IVF conceived pregnancies and 420 age and parity matched women with spontaneous pregnancies. All participants self-reported sociodemographic and pregnancy-related information. IVF treatment data were obtained after scrutinization of the medical records. The outcome, NVP at GW 17, was divided into: 1) absence of NVP, 2) NVP not requiring medications and 3) NVP requiring medications. NVP was then studied in relation to exposure and to different IVF treatment-associated variables.

Main results and the role of chance: The mean age of the participants was 33.7 years with 2/3 of the participants being primipara. IVF pregnant women reported more frequently comorbidities (such as hypertension, diabetes, migraine etc) (59.1% vs 49.9%), but less frequently alcohol consumption (38.4% vs 48.7%) compared to women with spontaneous pregnancies. Clinical and sociodemographic characteristics such as education, employment, smoking habits, maternal BMI, depression history, delivery fear and newborn gender, were otherwise similar between the groups. NVP with or without medications was not associated with mode of conception (p=0.889); 11.4% of women who conceived through IVF suffered from NVP requiring medications and 62.4% from unmedicated NVP *vs* 10.8% and 64.3% respectively of women with spontaneous pregnancies. Absence of NVP was reported by 26.2% of IVF and 24.9% of spontaneously pregnant women. However, in a subgroup analysis in the group of women who conceived through IVF, NVP was more frequently seen in the group who received cleavage stage embryos *vs* blastocysts (p=0.019). We could not however find any significant difference in the rate of NVP with or without medications between fresh (69.4%) or frozen/thawed embryo transfers (78.5%), nor between IVF(72.3%) and intracytoplasmic sperm injection(ICSI)(77.4%) treatments. Lastly, there was no significant difference between infertility diagnosis and NVP.

Limitations, reasons for caution: The study had limited power to detect differences in NVP in relation to mode of conception. In addition, there was a missing rate of 30.5% in the reported embryo stage variable. Finally, the rate of blastocyst-transfers during that period was lower than otherwise expected with current statistics.

Wider implications of the findings: It is still unclear whether IVF has an impact on the risk of NVP. However, transfer of a blastocyst may decrease the risk of developing NVP.

Trial registration number: Non- applicable

P-780 Natural cycles in frozen-thawed embryo transfer are associated with lower risks of preeclampsia and large-for-gestational age infants than artificial cycles; A systematic review and meta-analysis

J.A. Moreno[1,2], M.A. Checa[1,3], J.J. Espinós[1,3]

[1]Universitat Autonoma de Barcelona, Gynecology and Obstetrics, barcelona, Spain ; [2]Clinica de la Mujer, Reproductive Medicine, Vina del Mar, Chile ; [3]Fertty, Reproductive Medicine, Barcelona, Spain

Study question: Is Natural cycle frozen embryo transfer (NC-FET) safer than Artificial cycle frozen embryo transfer (AC-FET) for perinatal and maternal outcomes?

Summary answer: Women undergoing NC-FET have a lower risk of hypertensive disorders in pregnancy, preeclampsia and have a newborn large for gestational age than women after AC-FET.

What is known already: Frozen embryo transfer (FET) is associated with a higher risk of hypertensive disorders in pregnancy (HDP), preeclampsia, have a newborn large for gestational age (LGA) and macrosomia compared with fresh embryo transfer (ET), but it is hypothesized that the hormonal endometrium preparation used for the thawed embryo transfer may contribute to the development of placenta-related pregnancy complications.

Study design, size, duration: We conducted a systematic review and meta-analysis of 6 published cohort studies (including 66391 women)

Participants/materials, setting, methods: Literature searches were conducted to retrieve studies which reported on perinatal and maternal outcomes of pregnancies after FET. Databases searched included PubMed/Medline, SCOPUS and The Cochrane library to identify all relevant studies published until October 2019. Six studies matched the inclusion criteria.

Main results and the role of chance: NC-FET is associated with a lower risk of HDP (OR 0.66; 0.53-0.82), preeclampsia (OR 0.51; 0.41-0.64) and LGA

(OR 0.92; 0.87-0.97) compared to AC-FET. There was no significant difference in the risk of macrosomia. Regarding the secondary outcomes, NC-FET had lower odds of preterm birth (OR 0.87; 0.81-0.93), post-term birth (OR 0.41;0.21-0.83), low birth weight (OR 0.84; 0.79-0.89), small for gestational age (OR 0.91; 0.84-0.98), cesarean section (OR 0.75; 0.63-0.89), postpartum hemorrhage (OR 0.36; 0.30-0.42) and placenta accreta (OR 0.17; 0.09-0.32) compared with AC-FET. No statistical differences were noted in the remaining secondary outcomes. The included studies scored well on the Newcastle-Ottawa quality assessment scale.

Limitations, reasons for caution: The review is limited by the quality of the included studies and no randomized controlled trials (RCT) were included. Outcomes such as HDP and macrosomia showed substantial heterogeneity.

Wider implications of the findings: Concerning safety, NC-FET significantly decreases the risk of HDP, preeclampsia, LGA, preterm birth, post-term birth, low birth weight, small for gestational age, cesarean section, postpartum hemorrhage and placenta accreta. Further RCTs that addresses the effect of NC-FET and AC-FET on maternal and perinatal outcomes are warranted.

Trial registration number: not applicable

P-781 Is there a relationship between morphokinetic parameters and perinatal outcome? An analysis of singleton live births after single fresh embryo transfer.

A. Wertheimer[1], S. Onit[1], H. Alyssa[1], B.H. Avi[1], O. Galia[1], S. Ekaterina[1], A. Eran[1], S. Tzippy[1], S. Yoel[1]

[1]Helen Schneider Hospital for Women- Rabin Medical Center-Beilinson Hospital-, IVF and Infertility Unit, Petach-Tiqva, Israel

Study question: Is there a relationship between morphokinetic time-lapse parameters and the perinatal outcome in singleton gestations?

Summary answer: After adjusting for confounding variables, obstetric and perinatal outcomes were not related to any morphokinetic parameter.

What is known already: An association between embryo morphokinetics, implantation and clinical pregnancy rates was studied and reported in numerous publications. However, little is known regarding the association between morphokinetic parameters and the obstetric and perinatal outcomes and complications**.**

Study design, size, duration: A cohort study reviewing fertility and delivery files of all births from successfully treated infertile patients, who underwent ovarian stimulation, IVF embryo culture in time-lapse monitoring incubators and fresh single embryo transfer in a tertiary medical center between June 2013 and April 2019. The study group consisted of 177 single embryo transfers that resulted in 177 live births. Morphokinetic parameters were analyzed in correlation to perinatal complications.

Participants/materials, setting, methods: Embryos were annotated using a time-lapse system after ICSI or insemination. Embryo selection was based on locally established annotation criteria and the KID score provided by the Embryoscope^{tm} (Vitrolife, Denmark). The perinatal complications investigated were: gestational diabetes mellitus (GDM), intra-uterine growth restriction (IUGR), preterm labor (PTL), pre-eclamptic toxemia (PET), revision of uterine cavity, and post-partum hemorrhage (PPH). Logistic regression analysis was utilized to adjust results for potential confounders.

Main results and the role of chance: All deliveries in the study group resulted in live-births. Maternal baseline and treatment characteristics were similar between the groups except for a lower BMI in the IUGR complicated group and a higher BMI in the uterine revision complicated group (21.4±5.9 Kg/m^2 vs. 24.1±3.3 in the non-IUGR group, p=0.024, and 24.1±5.9 vs. 21.1±3.2 in the non-revision group, p=0.043).

Early morphokinetic time-lapse parameters including pronucleus fading, cleavage timings (t2-t8), second cell cycle duration (T3-T2) synchrony (T4-T3) and interval between 8 and 5 cells (T8-T5) were similar between the groups. Late morphokinetic parameters including full compaction at morula stage (tM), start of blastulation (tSB), full blastocyst (tB), and hatching (tHB) were similar between the groups as well, except for a shorter tB in the IUGR complicated group (96.7±5.3 hours vs. 103.2±3.1 in the non-IUGR group, p=0.020). Known implantation data of day 3 and 5 (KID3 and KID5 respectively) analysis revealed higher KID3 score in the IUGR complicated group (4.83±0.39 vs. 4.50±1.0 in the non-IUGR group, p=0.027). On multivariate regression analysis, none of the morphokinetic parameters that were significantly associated with perinatal complications in the univariate analysis were found to be significantly correlated with any of the perinatal complications.

Limitations, reasons for caution: Retrospective design, sample size and the inclusion of only singletons born after single embryo transfer.

Wider implications of the findings: Our findings may be used to reassure women and physicians that different morphokinetic parameters of the embryo transferred, providing the pregnancy continues to the third trimester, do not appear to be associated with increased risks for adverse obstetric and perinatal outcomes.

Trial registration number: not applicable

P-782 Trends in Ovarian Hyper-Stimulation Syndrome (OHSS) hospitalization rates in the United States (U.S.) based on 12,608,757 admissions in women aged 18-43 years: An ongoing concern

K. Rotshenker Olshinka[1], A. Badeghiesh[2], A. Volodarsky-Perel[3], N. Steiner[3], E. Suarthana[4], M.H. Dahan[5]

[1]McGill University- Montréal Quebec- Canada., Department of Obstetrics and Gynecology- Division of Reproductive Endocrinology and Infertility-, Montreal, Canada ;

[2]McGill University- Montréal Quebec- Canada., Department of Obstetrics and Gynecology, Montreal, Canada ;

[3]McGill University- Montréal Quebec- Canada., Department of Obstetrics and Gynecology- Division of Reproductive Endocrinology and Infertility, Montreal, Canada ;

[4]McGill University- Montréal Quebec Canada., Department of Obstetrics and Gynecology, Montreal, Canada ;

[5]McGill University- Montréal Quebec Canada., Department of Obstetrics and Gynecology- Division of Reproductive Endocrinology and Infertility, Montreal, Canada

Study question: Do we see downward trends in OHSS admissions since the development of preventative strategies, based on the National-Inpatient-Survey 2004-2014?

Summary answer: A decrease in OHSS hospitalizations, as well as number admitted-per-fresh IVF cycles, was seen 2004- 2008. However this decrease stopped and has plateaued since 2008.

What is known already: Many techniques have developed over the last 25 years to decrease OHSS risks. These include GNRH-antagonist IVF cycles, GNRH-agonist triggering of oocyte maturation and use of dopamine agonists to decrease VEGF production. Though uncommon, OHSS may sometimes be unpredictable, occurring in low risk women. Concurrently, use of assisted reproduction technology(ART) is increasing putting more women at risk for OHSS. In light of new techniques, It could be hypothesized that rate of OHSS admissions would decrease. However, according to the U.S. National ART Surveillance System (NASS), the rate of OHSS didn't change significantly between 2000-2009, estimated at 106/10,000 IVF cycles yearly.

Study design, size, duration: We conducted a retrospective population-based study utilizing data from the Health Care Cost and Utilization Project-Nationwide Inpatient Sample database (HCUP-NIS) over 11 years- 2004-2014, and based on the number of fresh IVF cycles per year as reported by the NASS during the study period. HCUP-NIS is the largest inpatient database in the U.S. covering over 97% of the U.S. population and represents approximately 20% of all discharges from community hospitals.

Participants/materials, setting, methods: We evaluated OHSS admissions between 2004 and 2014 inclusively. Women age 18-43 were queried for OHSS admissions using ICD-9 code 256.1.This resulted in a database of 12,608,757 admissions, 1900 for OHSS. Data was compared as absolute numbers, percentage of admissions per year in women 18-43 years old and as a percentage of all IVF cycles yearly, reported to the national database. Analysis was done using Mann-Kendall trend test (two-tailed).

Main results and the role of chance: During the study period, there were between 1.2-2/10,000 OHSS hospitalizations in women age 18-43, corresponding with a total of 1900 OHSS hospitalizations out of 12,608,757 database hospitalizations in the age group. The mean number of OHSS admissions per year during 2004-2007 was 261.5 (245-300, SD-25.8), and decreased to 171 subsequently (157- 191, SD-14.2). Evaluating data during the study period, there has been a decrease in both total number of OHSS hospitalizations (p=0.004),

and the frequency of OHSS admissions per 10,000 total women admissions (p=0.035). However, this decline was demonstrated only between 2004 and 2008. Since 2008, the total number of OHSS admissions per year (p=0.734) as well as OHSS admissions-per 10,000 women's admissions (P=0.733) have plateaued, with insignificant changes between 2008-2014. The number of fresh IVF cycles as reported by the NASS have increased from 94,242 to 128,268 cycles, during the study period. When dividing the number of OHSS hospitalizations in the database by the number of fresh IVF cycles, per year, we demonstrate similar trends, a decrease in the number of OHSS hospitalizations per IVF cycle, per year, during the study period (p=0.002) but a plateau from 2008-2014 (p=0.308).
Limitations, reasons for caution: The HCUP-NIS is the largest inpatient sample database in the U.S, but does not include non-community based hospitals, potentially excluding many OHSS admissions. Furthermore, the information was not gathered prospectively and is based on hospital records according to the ICD-9 code, which may mask bias.
Wider implications of the findings: OHSS remains a concern, as frontline ER physicians continued to see similar frequencies of OHSS-visits from 2008-2014. The financial burden of OHSS-hospitalizations persists. Although, techniques have likely resulted in a decrease in OHSS admissions since 2004, this has plateaued, and efforts to further reduce OHSS must continue.
Trial registration number: N/A

P-783 The best choice for embryo transfer in good prognosis patients undergoing freeze-only police: one plus one is better than two

P.A. Monteleone[1,2], P. Peregrino[2,3], J. Miorin[3], A. Gomes[4], H. Martin[2,4], T. Bonetti[5,6]

[1]Centro de Reproducao Humana Monteleone, Clinical director, Sao Paulo / SP, Brazil ;
[2]Universidade de São Paulo, Disciplina de Ginecologia - Departamento de Obstetrícia e Ginecologia, São Paulo, Brazil ;
[3]Centro de Reproducao Humana Monteleone, Clinical, Sao Paulo / SP, Brazil ;
[4]Centro de Reproducao Humana Monteleone, Embriology, Sao Paulo / SP, Brazil ;
[5]Universidade Federal de São Paulo, Gynecology, São Paulo, Brazil ;
[6]Centro de Reproducao Humana Monteleone, Scientific department, Sao Paulo / SP, Brazil

Study question: Which is the best choice for embryo transfer in good prognosis patients undergoing freeze-only police to get best pregnancy success rate and lower adverse effects?
Summary answer: In freeze-only cycles, transferring embryos one-plus-one in subsequent transfers is better than double embryo transfer regards both, higher ongoing pregnancy rates and lower multiple gestation.
What is known already: The freeze-only practice has been increasing recommended in ART. It is supposed to support better synchrony between embryo and endometrium in the absence of ovarian stimulation and is beneficial for patients at ovarian hyperstimulation syndrome (OHSS) risk or undergoing preimplantation genetic testing for aneuploidy. Single embryo transfer (SET) is the best choice to reduce multiple pregnancies and the associated risks, and have been recommended in ART, as well. Then, we hypothesized that the sequential frozen-thawed SET in freeze-only cycles of patients at OHSS risk could result in a more efficient approach compared to transfer of two embryos.
Study design, size, duration: Retrospective cohort study reviewed 5156 cycles performed between 2011 and 2019 in a private ART center. Five hundred and six (n=506) cycles match the inclusion criteria: ICSI using own oocytes and ejaculated/epididymal sperm, no embryo genetic diagnosis, freeze-only police (no fresh embryo transfer) and elective frozen-thawed embryo transfers (eFET) of one or two embryos in a subsequent hormone replacement cycle. eFET was defined as cycles which at least one surplus embryo was available after transfer.
Participants/materials, setting, methods: Patients underwent IVF cycle as routine and were split into two groups: elective Double Embryo Transfer (eDET:n=291) and elective Single Embryo Transfer (eSET:n=209). In the eSET group, 60 women who failed in the 1st eSET, had a 2nd transfer of one embryo. The ongoing pregnancy rates (OPR) after eDET versus eSET+SET were compared. The estimated cumulative ongoing pregnancy rate for eSET+SET group was calculated by [OPR at 1steSET+OPR at 2ndSET * (1-OPR at 1steSET)].
Main results and the role of chance: Groups were homogeneous to demographics characteristics as age (eDET: 34.9±4.2 versus eSET: 35.4±6.9; p=0.155), basal FSH measurement (eDET: 6.3±3.2 versus eSET: 6.3±5.0; p=0.599), dose of gonadotropin administered (eDET: 1869.9±481.4 versus eSET: 1809.4±372.2; p=0.129). Despite of MII oocytes recovered had been higher in eDET (13.3±7.4) versus eSET (15.2±8.5; p=0.008), the number of embryos vitrified and available for transfer (eDET: 8.8±4.5 versus eSET: 9.1±4.5; p=0.381) were similar between groups. After the 1st eFET, the OPR was statistically similar between groups (eDET: 46.9% versus eDET: 44.4%; p=0.596). When we evaluated the estimated cumulative OPR after a 2nd FET in the eSET+SET group, it was significantly higher (63.5%) than eDET (46.9%, p<0.001). Additionally, while the eSET+SET group had 2.2% of multiple gestation, the eDET group had 26.9% of multiples (p<0.001). The choice about the number of embryos to be transferred was performed as a shared decision-making process between patients and doctors, after an explanation of advantages and disadvantages of each situation as routine.
Limitations, reasons for caution: The retrospective characteristic of this study is a limitation. Another point to be considered is that quality of embryos transferred were not assessed in this study. However, all transfers included were elective, which indicate that the best quality embryo was always preferred.
Wider implications of the findings: We demonstrated that the transfer of embryos one by one results in higher cumulative ongoing pregnancy rate compared to a transfer of two embryos in one cycle, and reduce drastically the multiple pregnancy. Thus, we conclude the transfer of one by one is the best choice in freeze-only cycles.
Trial registration number: not applicable

P-784 Residents in training and senior physicians have comparable oocyte retrieval rates, but physicians perform significantly better in complicated cases.

C. Verhaeghe[1], H. El Hachem[2], R. Corroenne[1], C. Dreux[1], P. Jeanneteau[1], L. Inchboard[3], P. May-Panloup[1], P.E. Bouet[1]

[1]Angers University Hospital, Department of Reproductive Medicine, Angers, France ;
[2]Clemenceau Medical Center, Obstetrics & Gynecology- Reproductive Endocrinology & Infertility, Beirut, Lebanon ;
[3]Angers University Hospital, Clinical Research Center, Angers, France

Study question: Is there any difference in oocyte retrieval rates (ORR) between residents in training and senior physicians?
Summary answer: Residents in training and senior physicians have comparable ORR. Operator experience makes a significant difference in cases of endometriosis and polycystic ovarian syndrome (PCOS).
What is known already: Most university hospitals in France do not have a simulation based training program for oocyte retrievals (OR) for residents in training. They usually learn by first assisting senior physicians in the operating room, before gradually performing them, starting with one ovary (usually the more accessible one) (mixed retrievals), then the whole procedure. This approach has proven to be somewhat efficient, but there is a large variability in the learning curve of residents, with one study showing that anywhere between 17 and 50 OR are needed to achieve clinical proficiency.
Study design, size, duration: We performed a retrospective single center study at the Angers university hospital between May 2017 and September 2018. All oocyte retrievals performed during that time (n=690) by all the senior physicians (n=5) and the Obstetrics and Gynecology residents in training (n=7) were included.
Participants/materials, setting, methods: We defined the ORR as the rate of oocytes retrieved to the number of punctured follicles (at our center, all follicles ≥8 mm are punctured). Before the retrieval, each participant counted the total number of follicles to be punctured. The number of follicles punctured per aspiration tube and the name of the operator were written on a sheet that was annexed to the tube.
Main results and the role of chance: Overall, 674 OR were included for the final analysis, 315 performed by residents, 220 by senior physicians, and 139 by both residents and physicians (one side per operator). There was no significant difference in the mean ORR between residents (61.9%) and senior physicians (62%) (p=0.99). Multivariate analysis showed a non-significant difference of 2% in the performance level between the two groups (p=0.52). In cases of endometriosis, there was a significant difference of 12% in favor of senior physicians (p=0.03). Finally, in women with PCOS,

there was a significant difference in the ORR between residents (50%) and senior physicians (56%) (p<0.001), but both groups had suboptimal ORR (defined as <60%).

Limitations, reasons for caution: The main limitation of our study is the retrospective design, and the lack of randomization of which ovary to be punctured between residents and physicians in mixed retrievals.

Wider implications of the findings: Residents in training and senior physicians have overall comparable mean ORR, but senior physicians perform significantly better in cases of endometriosis and PCOS. Therefore, a more targeted training, with the help of simulation training, should be introduced to improve the performance levels in these more challenging cases.

Trial registration number: N/A

P-785 Cardiac diastolic function of 8-9-year-old singletons following fresh or frozen embryo transfer compared to naturally conceived – preliminary results from a cardiac magnetic resonance study

I. Mizrak[1,2], L.L. Asserhøj[1,3], R.B. Jensen[3], M.A.V. Lund[4], G.F. Heldarskarð[1,3], A.S.L. Kjær[1,3], G. Greisen[5], N. Vejlstrup[6], P.L. Madsen[2], A. Pinborg[1]

[1]Rigshospitalet, Fertility Clinic, Copenhagen, Denmark ;
[2]Herlev-Gentofte Hospital, Department of Cardiology, Herlev, Denmark ;
[3]Rigshospitalet, Department of Growth and Reproduction, Copenhagen, Denmark ;
[4]University of Copenhagen, Department of Biomedical Sciences, Copenhagen, Denmark ;
[5]Rigshospitalet, Department of Neonatology, Copenhagen, Denmark ;
[6]Rigshospitalet, Department of Cardiology, Copenhagen, Denmark

Study question: Do singletons conceived after fresh or frozen embryo transfer (FET) have altered cardiac diastolic function compared to naturally conceived children at age 8-9 years?

Summary answer: Children conceived after fresh embryo transfer (Fresh ET) may have impaired left ventricle peak-filling-rate compared to the other two groups.

What is known already: Epigenetic changes secondary to assisted reproductive technology (ART) may alter the cardiovascular system. While singletons conceived after FET are more likely to be large-for-gestational-age, singletons from Fresh ET are at increased risk of being small-for-gestational-age. Studies have suggested that ART children compared with NC children more often have endothelial dysfunction, exaggerated pulmonary hypoxic vasoconstriction, insulin resistance, higher body mass index and arterial blood pressure. In patients known with metabolic syndrome, the latter cardiovascular changes are associated with cardiac diastolic dysfunction. So far, no studies have addressed the effect of Fresh ET and FET on diastolic cardiac function**.**

Study design, size, duration: In all 150 singletons are planned to be recruited in this cohort study. We perform cardiac MRI in 50 children conceived after Fresh ET; 50 children conceived after FET and 50 NC singletons. So far, 124 children have undergone cardiac MRI, and images from 59 children have been analyzed with respect to diastolic function in a blinded manner. The inclusion started in January 2019 and will be completed in March 2020.

Participants/materials, setting, methods: ART singletons were identified through the Danish IVF and Medical Birth registries. NC children matched with respect to sex and birth year served as controls. Cardiac MRI was performed without anesthesia or contrast. Left (LV) and right ventricular (RV) function and volumes, maximum volume of left atrium (LA), LA early-emptying-fraction, LV peak-ejection-rates (PER), peak-filling-rates (PFR) and PER/PFR are reported. Variables were compared by ANOVA or chi-square test (P≤0.05, double-sided) without correction for multiplicity of P.

Main results and the role of chance: Presented results are from preliminary data as inclusion and post-processing work is still ongoing. We have cardiac MRI data on RV and LV systolic function and LV diastolic function from 59 children: 20 children conceived after Fresh ET; 20 children conceived after FET and 19 NC children. The median age of the participants is 9.0 (IQR 8-9) years. Data show no differences in LV and RV ejection fraction, end-diastolic and end-systolic volumes, stroke volumes and left ventricular mass between the three groups. With respect to data on LV diastolic function, no differences were observed in LA volume and LA early emptying fraction; but children conceived after Fresh ET tended to have lower PFR (292± 63 mL/s *vs*. 338±46 mL/s (FET) and 305±78 mL/s (NC); P=0.066; mean±SD) and higher PER/PFR ratio (1.06±0.27 *vs*. 0.91±0.19 (FET) and 0.95±0.17 (NC); P=0.087) compared to the other two groups.

Limitations, reasons for caution: Results are based on preliminary analysis on a cohort. Further analysis of cardiac diastolic function will include peripheral resistance and artery distensibility. Selection bias may be seen as the participation rate was 19.1% in Fresh ET, 21.7% in FET and only 9.1% in NC children.

Wider implications of the findings: The trend towards a decreased LV PFR in the Fresh ET group could be related to the endothelial dysfunction and increased arterial blood pressure previously described after ART. If the absence of diastolic dysfunction in FET children will be confirmed in the full study group, this would be reassuring.

Trial registration number: NCT03719703

P-786 Comparison of physical growth parameters of children conceived by donor oocytes with fresh versus frozen embryo transfer upto 5 years of age :a prospective study

M. Varma[1], S. Singh[2], S. Verma[3], H. Tuli[4], R. Kumar[4], T. Kaur[5]

[1]Sadbhavna Medical & Heart Institute, OBGY, Patiala, India ;
[2]Artemis Health Institute, Reproductive Medicine, Gurugram, India ;
[3]University of California, Life Sciences, San Diego, U.S.A. ;
[4]Sadbhavna Medical & Heart Institute, Neonatology, Patiala, India ;
[5]Sadbhavna Medical & Heart Institute, Anaesthesia, Patiala, India

Study question: Are the weight/length/height/head circumference (mean Z-scores)different in children conceived by frozen embryo transfer(FET) or freshET in a donor oocyte(DO) model, during the first 5 years

Summary answer: Weight of children at 2-5 years was significantly more in FET than fresh ET group with DO after adjusting for maternal age and BMI

What is known already: Prospective studies regarding the potential effect of freezing protocols on childhood growth are limited with comparable / trends of increased weight. FreshET has been compared to FET where ovarian stimulation remains an important variable affecting early events in pregnancy. DO babies have been reported to be spared of macrosomia in FETs. In this study, comparing the growth of children conceived by fresh ET versus FET, in a DO model, with similar laboratory procedures and vitrification protocols, a better insight into the effects of vitrification is observed, as several important confounders are eliminated. There is limited published data regarding the same.

Study design, size, duration: In this prospective cohort study, conducted from 2014-2019 at a tertiary centre, 209 children conceived by DO (delivered after 32 weeks gestation) were followed-up at birth and at one point of time (age 1month – 5 years) Data was available for mothers(n=161), children(n=189); conceived by freshET (n=109),FET(n=80). Both groups had similar ICSI, vitrification/thawing, ET(2/3 cleavage stage) protocols. The IVF program, deliveries, follow-up was conducted at the same institution. Some children were followed-up by post

Participants/materials, setting, methods: Weight/length/height/head circumference(HC) were recorded at birth and later at one point of time as Z-scores (WHO growth standards) to adjust for age at measurement. This was further adjusted for maternal age and BMI. Children were divided into two groups < 2 years and 2-5 years. Singletons were analysed as a sub-set. Two-sample t- test was used to compare means. Multiple regression analysis was used to adjust for potential confounders. Statistical significance was set at *P*<.05(2-tailed)

Main results and the role of chance: Maternal mean age and BMI were comparable in the fresh ET and FET groups at 35.30 ± 5.38 years versus 33.99± 6.65 years(*P*=.13) and 26.70±1.90kg/m2 versus 26.60± 1.99kg/m2(*P*=.72),respectively.

All mothers were non-smokers, 150/161 were nulliparas Incidence of pregnancy complications like preeclampsia, gestational diabetes was similar in both the groups. Oocyte donors were< 30 years with comparable mean BMI(*P*=.34)

The mean Z -scores for birth weight of singletons(n=133) was comparable for the fresh(n=68) and frozen transfers(n=65) after adjusting for maternal age and BMI(*P*=0.06)

In the <2 years cohort, children conceived by fresh ET(n=34) and FET(n=41) and in the 2-5 years group, fresh ET(n=75) and FET(n=39).

In the 2-5 years cohort, mean Z -scores for weight was significantly more for the FET group of all children, compared to the fresh ET group (0.45±1.18 versus -0.09±1.15 ,*P*=.02) Even in the singleton subset, the weight of children in the FET group was more versus fresh ET (0.53±1.18 versus -0.10 ±1.09, *P*=.01). This difference remained significant in both groups even after adjusting for maternal age and BMI(*P*=.004 and *P*=.015,respectively).

Mean Z -scores for weight in the <2year groups, as well as, length/height in all the groups were comparable. HC did not follow any definite statistically significant trend.

Limitations, reasons for caution: This was a single centre study with a limited sample size. After birth, the growth parameters of children were recorded at a single point in time only (requiring the need for Z -scores for comparison)

Wider implications of the findings: Our study, using the DO model, shows significant greater weight of children at 2-5 years conceived by frozen compared to fresh ET. This needs to be confirmed in larger studies with longer follow-ups. The mechanism and clinical relevance of this, also needs to be scientifically explored

Trial registration number: Not Applicable

P-787 Effect of embryo cryopreservation duration on pregnancy-related complications and birthweight after frozen-thawed embryo transfer: a retrospective cohort study

X. Jing-Jing[1], L. Chen[1], C. Li[1], H.F. Huang[1], Y.T. Wu[1]

[1]School of Medicine- Shanghai Jiao Tong University, International Peace Maternity and Child Health Hospital, Shanghai, China

Study question: Whether embryo cryopreservation duration has impact on pregnancy-related complications and neonatal birthweight in women with frozen-thawed embryo transfer (FET)?

Summary answer: Embryo cryopreservation duration does not have negative effects on pregnancy-related complications or birthweight after FET.

What is known already: Several studies have indicated that embryo cryopreservation duration has no effect on the survival rate of the embryo after thawing or the neonatal birthweight in singleton. However, almost all these studies excluded the multiple pregnancies, and no study has focused on the effect of embryo cryopreservation duration on maternal safety in terms of pregnancy-related complications.

Study design, size, duration: A retrospective cohort study including all FET cycles with livebirth deliveries in a university hospital from May 2010 to September 2017 was conducted. These deliveries were grouped according to the cryopreservation duration of the transferred embryo (≤3 months, 4-6 months, 7-12 months, and >12 months).

Participants/materials, setting, methods: Among all 12,158 FET cycles, a total of 3,864 livebirth deliveries comprising 2,995 singletons and 1,739 multiples were included in the analysis. Multinomial and multilevel logistic regression were used to evaluate the associations between embryo cryopreservation duration and pregnancy-related complications or neonatal birthweight among the groups stratified by singletons and multiples. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated and adjusted for potential confounding factors.

Main results and the role of chance: Compared with women undergoing FET after a cryopreservation duration within 3 months, women undergoing FET after a cryopreservation time longer than 3 months did not show any increased risk of gestational diabetes mellitus, gestational hypertension, preeclampsia, meconium staining of the amniotic fluid, preterm birth or cesarean section deliveries in singletons. For multiples, the risk of meconium staining of the amniotic fluid was found significantly increased for women transferred embryo of over 12 months cryopreservation (adjusted OR=2.22, 95%CI: 1.24-3.94), while the risk of other pregnancy-related complications was comparable among groups. Furthermore, the risk of lower birthweight, macrosomia, small-for-gestational-age, or large-for-gestational-age for either singletons or multiples was not affected by long-term cryopreservation.

Limitations, reasons for caution: Because some patients were concerned about the adverse effects of extremely long-term embryo cryopreservation, the study population on this part was lacking in this study. All embryos transferred in this study were cryopreserved via vitrification, so, our results only applied to women with FET cycles using vitrification rather than slow-freezing.

Wider implications of the findings: This was the first study investigating the safety of transferring long-term cryopreserved embryos in terms of pregnancy-related complications in both singleton and multiple pregnancies. However, further studies with long-term follow-up are still required to assess the possible effects of long-term cryopreservation on child growth and development.

Trial registration number: not applicable

P-788 Incidence of multiple births in relation to current regulations in Turkey regarding the number of embryos transferred

I.N. Duzguner[1], S. Kahraman[1], S. Duzguner[1], Y. Sahin[1], F. Sagır[1], K.B. Yuksel[1], U. Gokturk[1]

[1]Istanbul Memorial Hospital Assisted Reproductive Technologies and Genetics Center, Assisted Reproductive and Genetics, Istanbul, Turkey

Study question: What are the consequences of current regulations in Turkey in relation to incidence of multiple births?

Summary answer: Varying interpretations of current regulations can result in a high risk of multiple births.

What is known already: Before 2010, there was no regulation in Turkey regarding the number of embryos to be transferred. In March 2010, regulations were introduced restricting this number to one, except in particular cases: firstly, up to the maternal age of 35, single embryo transfer (SET) only is allowed for the first two attempts, but double embryo transfer (DET) is allowable for the third or any further cycles. From the age of 35 onwards, a maximum number of two embryos may be transferred in any one cycle. Consequently, many patients have understood this as a right to have 2 embryos transferred.

Study design, size, duration: This retrospective, single center study evaluated single versus multiple births in 5918 fresh and frozen thawed blastocyst transfer cycles and carried out in compliance with current regulations between 2014-2018.

Participants/materials, setting, methods: The study is based on data from İstanbul Memorial Şişli Hospital, ART Center regarding two groups of patients as defined in Turkish regulations. Clinical results of fresh and frozen-thawed single or double blastocyst transfer with 2,3 or 4 previously ART attempts in patients <35 years, SET (n=2562 cycles) and DET (n=662 cycles) and ≥ 35 (35-42) years SET (n=1930 cycles) and DET (n=764 cycles) were analysed.

Main results and the role of chance: As a result of the wording of the current regulations there was a high demand for DET. Furthermore, in these cases multiple birth rates were high at 49.4% (174/352) in the DET group of patients <35 years and 27.6% (82/297) in the DET group of patients ≥ 35 years. There were no statistical difference for live birth rate between blastocyst SET and DET in patients <35 (51.9%- 53.1%; p:0,32) and in patients ≥ 35 (44.4% - 38.4%; p:0,09). Current regulations can act as an encouragement to a demand for DET and should therefore be amended to encourage the use of single blastocyst transfer. The use of single blastocyst transfer should be maximized for women under 35. For women aged 35-42, transfer of a euploid single embryo should be considered as an alternative to DET.

Limitations, reasons for caution: The study was a retrospective analysis.

Wider implications of the findings: Regulations in all countries need carefully evaluation and amendment as necessary to reduce the incidence of multiple births in fresh and frozen-thawed embryo transfer cycles.

Trial registration number: Not applicable

P-789 Importance of long-term follow-up of ART derived babies not only for monitoring physical development, but also to detect latent congenital malformation.

A. Obashi[1], H. Matsumoto[1], S. Mizuno[1], A. Fukuda[1], Y. Morimoto[2]

[1]IVF Osaka Clinic, Department of Obstetrics and Gynecology, Higashi-osaka, Japan ; [2]HORAC grand front osaka clinic, Department of Obstetrics and Gynecology, Osaka, Japan

Study question: It is not clarified yet if ART affects long-term physical development of the resulting babies.

Summary answer: Long-term follow-up of ART babies indicated that babies by ART were not significantly different from the babies without ART, but detected latent congenital anomalies afterward.

What is known already: There is no increase of congenital anomalies in ART neonate compared to natural pregnancies based on the official data of JSOG (Japan Society for Obstetrics and Gynecology). Moreover, physical development of ART derived babies is within 50 percentiles of national average from the data collected by Ministry of Health, Labor and Welfare of Japan (MHLW). However, most of the developmental reports followed the babies until 2 years old, worldwide.

Study design, size, duration: The present investigation is retrospective cohort study. Total of 2641 babies, 1304 male and 1337 female, derived from ART procedures performed between January 2008 and December 2016 were evaluated for analysis. Physical developmental data were evaluated at ages of one and a half (637 babies), 2 years (521 babies), 3 years (360 babies), 5 years (160 babies) and 6 years (62 babies).

Participants/materials, setting, methods: Congenital anomaly rate diagnosed at birth was also compared to the national data provided by the Japan branch of International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR). Physical development was evaluated by weight, height and infant-child development index at ages of one and a half, 2 years, 3 years, 5 years and 6 years by comparing to standard developmental curve of Japanese infant-child by MHLW in each gender.

Main results and the role of chance: Congenital anomaly rate at birth of ART babies was 2.8% which was not different from 2.6% by Japan branch of ICBDSR. Mean with standard deviation of weight and height of male and female babies at birth, ages one and a half, 2, 3, 5 and 6 years were placed within 3rd and 97th percentile on standard curve given by MHLW. Median weight and height of both male and female babies at birth, ages of one and a half, 2, 3, 5 and 6 was plotted between the 25th and 75th percentile on standard curve as well. However, nine ART babies diagnosed normal at birth were newly diagnosed congenital anomaly afterward until age of 5.

Limitations, reasons for caution: Data from a single institution. Data analyzed on ART babies included normal and anomaly babies. Data analyzed only full-term infant. However, Gestational ages and maternal complication during pregnancies were disregarded.

Wider implications of the findings: Long term follow-up of ART babies until 6 years indicated that ART procedures were not detrimental, because their development index stayed within 25-75 percentile on Japanese standard without ART. However, the present study recognized the importance of long-term follow-up due to the existence of newly diagnosed congenital anomalies afterward.

Trial registration number: not applicable

P-790 Implementation of embryo transfer learning using medical simulation tool: comparison of two embryo transfer simulators

M. Ouazana[1], M. Bourdon[1], C. Maignien[1], K. Pocate[2], V. Blanchet[1], J. Gonnot[1], C. Cervantes[1], E. Laviron[1], C. Patrat[2], C. Chapron[1], L. Marcellin[1], P. Santulli[1]

[1]Centre Hospitalier Universitaire CHU Cochin-Bâtiment Port Royal, Department of Gynaecology Obstetrics and Reproductive Medicine Paris, Paris, France ; [2]Centre Hospitalier Universitaire CHU Cochin-Bâtiment Port Royal, Department of Histologie-Embryology and Reproductive Biology- Paris- France., Paris, France

Study question: Are students interested in the use of virtual simulator learning for embryo transfer (ET)?

Summary answer: Students expressed a great interest in ET simulator. Simulator for ET could help to improve the mastery of the equipment and skills for ET procedure.

What is known already: Embryo transfer is a main part for the obtainment of a pregnancy with assisted reproductive technology (ART). It is recommended to form practitioners for the ET technic to optimize pregnancy chance. Virtual reality training is proposed to improve clinician's skills, have a standardized technique and enhance ART outcomes.

Study design, size, duration: An observational study was conducted at the university-based reproductive medicine center of our institution during a medical training concerning infertility and ART in June 2019. Two ET simulators (Simulator A and Simulator B) were tested by trainees, supervised by experienced doctors. Inclusion criteria were (i) all the trainees attending the medical training, (ii) who answered the question survey concerning the two simulators A and B. Exclusion criteria was trainees that did not evaluated both simulators.

Participants/materials, setting, methods: The formation started with a 30 minutes' presentation of the theoretical basis to ET followed by training for ET procedures on the two simulators. Trainees were asked to use both, and the running order was randomly determined. The duration of the training was twenty minutes for each simulator. All student had to practice two type of ET with various difficulty with each simulator. Trainees received an email with a question-survey the night following the training.

Main results and the role of chance: During the study period, 32 trainees which have answered the evaluation for both simulators A and B were included. Used of simulators for ET teaching allows a reassurance for most of the trainees (81.3% in Simulator A and 75.0% in Simulator B group; p=0.55). Both simulators help to improve the mastery of the equipment (p=0.62) and the knowledge of the ET (p=0.19) without significant differences between groups. However, significant differences between simulators have been observed concerning the learning of precision of the ET procedure (p <0.01), the learning about the pathway to introduce the catheter into the cavity (p=0.04), the help for proper placement of the catheter into the uterine cavity (p=0.03) and the reproduction of the embryo into the uterine cavity (p=0.04) in favor of simulator A. Whatever the simulator used, trainees estimated that virtual simulator should be encouraged for learning the practice of ET.

Limitations, reasons for caution: In this preliminary study, we evaluate the interest of students for this type of training, consequently, we have no evaluation of long term benefit on pregnancy chances improvement. Moreover, simulator can't be similar to women's anatomy and isn't suited to reflect on hard cases.

Wider implications of the findings: In order to form and reassure practitioners, simulation learning for ET should be more widespread during gynecologic formation. Nevertheless, optimization of simulations tools for learning is needed.

Trial registration number: NA

P-791 Perinatal outcomes of assisted reproduction compared to natural conception in couples with unexplained subfertility. Follow up of two randomized clinical trials

J. Wessel[1], F. Mol[1], N. Danhof[1], A. Bensdorp[1], R. Tjon-Kon Fat[1], M. Mochtar[1], INeS study Group([1])[j.a.wessel@amsterdamumc.nl], SUPER study group([1])[j.a.wessel@amsterdamumc.nl], M. Van Wely[1]

[1]Amsterdam UMC- University of Amsterdam, Centre for Reproductive Medicine, Amsterdam, The Netherlands

Study question: Does the fertility procedure affect perinatal outcomes of singleton live births compared to natural conceptions in couples with unexplained subfertility?

Summary answer: Compared to natural conception, singletons born after IVF had comparable birth weights, while intrauterine insemination with ovarian stimulation (IUI-OS) resulted in lower birth weights.

What is known already: Children conceived by assisted reproduction have poorer perinatal outcomes such as lower birthweight (LBW) and a higher risk of premature birth (PTB) than naturally conceived children. This might be due to the assisted reproduction, such as laboratory procedures or the ovarian stimulation, or to an intrinsic factor in couples with subfertility. However, most studies compared perinatal outcomes in subfertile couples, carrying the risk of an inherit intrinsic factor, to fertile couples without such an intrinsic factor.

Study design, size, duration: We performed a follow up study of two randomized clinical trials performed in couples with unexplained subfertility, including the naturally conceived singletons. The primary outcome was birthweight. The secondary outcomes were LBW (defined as birthweight <2500g) and PTB (defined as delivery <37 weeks of gestation). We calculated differences in birthweight using regression analyses adjusted for maternal age, BMI, smoking, parity, duration of subfertility, child gender and preeclampsia (PE) during pregnancy.

Participants/materials, setting, methods: To investigate the impact of laboratory procedures on perinatal outcomes, we compared singletons

conceived by IUI-OS or IVF to naturally conceived singletons. To study the impact of ovarian stimulation we stratified the IUI group for type of stimulation (follicle stimulation hormone (IUI-FSH) or clomiphene citrate (IUI-CC)) and the IVF group in modified natural cycle (IVF-MNC) and in standard IVF with single embryo transfer (IVF-SET). We compared all groups to naturally conceived singletons.

Main results and the role of chance: In total there were 553 ongoing pregnancies, which resulted in 493 live birth singletons. Of the 493 singletons, 209 were conceived after IUI-OS (136 with FSH and 73 with CC as ovarian stimulation), 159 after IVF (50 after IVF-MNC and 109 after IVF-SET) and 125 were conceived naturally. Singletons conceived following IUI-OS had lower birthweights than naturally conceived singletons (adjusted difference -127.2gr, 95%CI -249.9 to -4.6). The difference was observed irrespective of whether FSH or CC was used (adjusted difference IUI-FSH -127.5, 95%CI -262.7 to 7.8; IUI-CC -124.7, 95%CI -286.8 to 37.3).

Birthweights from singletons conceived after IVF were comparable to birthweights after natural conception (adjusted difference -5.3, 95%CI -130.5 to 120.0). When corrected for ovarian stimulation no significant difference was found between IVF-MNC and IVF-SET compared to natural conception (adjusted difference IVF-MNC 78.7 95%CI -94.4 to 251.8; IVF-SET -44.4, 95%CI -181.4 to 92.7). We found no evidence of a difference in the secondary outcomes LBW and PTB.

Limitations, reasons for caution: The results are limited by the number of cases.

Wider implications of the findings: In unexplained subfertility, IUI-OS but not IVF might result in slightly compromised perinatal outcomes. Our findings suggest that a part of the previously observed compromised perinatal outcomes are due to an intrinsic effect in infertile couples. Further research in a larger population of singletons is necessary to substantiate our findings.

Trial registration number: NTR939 and NTR 4057

P-792 The impact of in-vitro fertilization treatment on the maternal renin-angiotensin-aldosterone-system in very early pregnancy

R. Wiegel[1], A.H.J. Danser[2], D. Aoulad Fares[1], E.A.P. Steegers[1], J.S.E. Laven[1], S.P. Willemsen[3], V.L. Baker[4], R.P.M. Steegers-Theunissen[1], F. Von Versen-Höynck[5]

[1]Erasmus Medical Center- University Medical Center, Obstetrics and Gynecology, Rotterdam, The Netherlands ;
[2]Erasmus Medical Center- University Medical Center, Internal Medicine, Rotterdam, The Netherlands ;
[3]Erasmus Medical Center- University Medical Center, Biostatistics, Rotterdam, The Netherlands ;
[4]Johns Hopkins University School of Medicine, Reproductive Endocrinology and Infertility, Baltimore, U.S.A. ;
[5]Hannover Medical School, Obstetrics and Gynecology, Hannover, Germany

Study question: What is the impact of in-vitro fertilization (IVF) treatment on associations between corpus luteum (CL) function and the maternal renin-angiotensin-aldosterone-system (RAAS) in early human pregnancy?

Summary answer: Maternal prorenin, renin and aldosterone levels differed significantly between spontaneous conceptions and those after IVF in early pregnancy which may be related to CL function.

What is known already: The RAAS is involved in the maternal cardiovascular and hemodynamic adaptation to pregnancy. Prorenin is synthesized and secreted during maturation of the ovarian follicles by the unique temporary endocrine structure, the CL. The absence of CL is associated with an increased incidence of preeclampsia. We hypothesize that this association can be explained by derangement of the maternal RAAS. The objective of this study is to investigate the potential impact of IVF treatment and CL status on component levels of the maternal RAAS in early pregnancy.

Study design, size, duration: We pooled two prospective cohorts with enrollment before 10 weeks of gestation: 75 pregnancies from the Stanford Pregnancy outcome after infertility (POFI) study and 202 pregnancies from the Rotterdam periconception cohort (Predict) conducted at the Erasmus University Medical Center. Pregnancies were stratified for IVF procedure and CL number, i.e. programmed cycle frozen embryo transfer [FET], n=28 (0CL), natural cycle FET, n=41 (1CL), fresh embryo transfer [ET], n=69 (>1CL) and spontaneous conceptions, n=139 (1CL).

Participants/materials, setting, methods: Prorenin and renin blood levels were measured in the Stanford study at 5 weeks gestational age (GA) and in the Predict study at 9 and 11 weeks GA, with additional aldosterone measurements, all carried out by the laboratory of the Erasmus Medical Center. Fertility treatment, type and dose of gonadotropins and fertility parameters depicted as number of follicles, retrieved oocytes and sum of follicle diameter during oocyte pick-up (OPU) were retrieved from medical records.

Main results and the role of chance: Maternal total renin, prorenin and renin were significantly lower in the absence of the CL at all-time points when compared to 1 CL (at 5 weeks: 1.9-, 2.2- and 1.7-fold lower and at 9 weeks: 2.4-, 2.5- and 2.4-fold lower, respectively). Maternal total renin at 5 weeks and total renin, prorenin, renin and aldosterone at 9 and 11 weeks were significantly higher in the presence of multiple CL when compared to 1CL (at 5 weeks: 1.5- and at 9 weeks: 1.4-, 1.4-, 1.2- and 1.8- fold higher, respectively). Stratified by IVF procedure the maternal total renin, prorenin, renin at all-time points and aldosterone at 11 weeks were significantly different, pregnancies conceived by fresh ET showing the highest RAAS component levels and with programmed cycle FET the lowest. Sum of follicle diameter prior to OPU was positively associated with maternal prorenin levels at 11 weeks GA (β: 0.002 [95%CI: 2.88e-05;0.0047]; p=0.048 after adjustment for gonadotropin type and dose, maternal age and parity). After ovarian stimulation and fresh IVF transfers, Menopur (urinary FSH containing some HCG) showed significantly higher renin and aldosterone levels during late first trimester when compared with Bemfola (recombinant FSH biosimilar), (p=0.009 and p=0.002) respectively.

Limitations, reasons for caution: Due to the observational character of this study residual confounding cannot be ruled out. Furthermore, patients were recruited from two tertiary referral hospitals with potential differences in fertility treatment. According to relatively smaller sample sizes after stratification by IVF procedure and CL number, our findings should be interpreted carefully.

Wider implications of the findings: This data suggests that IVF impacts the association between periconception ovarian biology and maternal RAAS activity. Since RAAS is involved in maternal cardiovascular adaptation to early pregnancy, modulation of RAAS might contribute to pregnancy complications. Future research should investigate the impact of IVF on ovarian biology and CL during pregnancy.

Trial registration number: NL6684

P-793 Validation of the competency and benchmark values of the Vienna Consensus for the ART laboratory performance indicators

G.H. Trew[1], C. Henry[2], S.M. Nelson[3]

[1]The Fertility PartnerShip, Faculty of Medicine, London, United Kingdom ;
[2]The Fertility Partnership, Information and Technical Division, Oxford, United Kingdom ;
[3]University of Glasgow, School of Medicine, Glasgow, United Kingdom

Study question: To assess whether the Vienna Consensus values regarding competency and validation for performance, reference and key performance indicators are appropriate for the proposed reference population.

Summary answer: The competency values can be achieved but the values for good blastocyst development are dependent on consistency of embryo grading, rather than culture conditions.

What is known already: The Vienna Consensus proposed competency and aspirational benchmark values for a range of performance indicator, reference indicator and key performance indicators for the laboratory. These were derived based on surveys, scientific evidence and personal clinical experience with the minimum and expected and target value based on 50 and 75 percentile values, respectively. With further discussion of each proposed indicator until consensus was reached. However, whether these values are appropriate or attainable is unknown and the group encouraged national and international bodies to gather data that can be used for the derivation of KPI standard values.

Study design, size, duration: Retrospective cohort study of 6,339 fresh treatment cycles and 4,670 frozen embryo transfers in women <40 years old; using their own fresh oocytes; ejaculated spermatozoa (fresh or frozen); no PGD/PGS (PGT); and having routine IVF or ICSI. All cycles were conducted within the UK between 1 January 2018 and 31 December 2019.

Participants/materials, setting, methods: Women undergoing assisted conception at the eight UK clinics of The Fertility Partnership who met the inclusion criteria suggested by the Vienna Consensus for reference value

derivation . Data was recorded in real-time for clinical and laboratory characteristics using IDEAS with data retrospectively analysed and visualised using Qlik.

Main results and the role of chance: Of the 6339 fresh cycles, the mean age was 33.9 years, with a mean of 12 oocytes retrieved with an overall pregnancy rate of 41.3% per embryo transfer, with a multiple pregnancy rate of 9.3%, while for the 4,670 frozen cycles the mean age was 34.1 years with a clinical pregnancy rate of 45% and multiple rate of 11.4%. Of the 72,586 oocytes retrieved 58.6% of cycles were fertilised by ICSI. The observed mean and (suggested competency Indicators noted in brackets) for all oocytes retrieved for follicles >10mm was 80.9% (80-95%), and 79.2% (75-90%) were MII. For the Performance Indicators; IVF polyspermy was 5.5% (<6%), 1PN IVF 3.64% (<5%), IPN ICSI 2.69% (<3%) and good blastocyst 24.56% (≥30%). For KPIs the ICSI damage rate was 6.7% (<10%), ICSI fertilisation rate 66.6% (≥65%), IVF normal fertilisation rate 61.2% (≥60%), IVF failed fertilisation rate 3.2% (<5%), cleavage rate 93% (≥95%), day 3 development rate 64.7% (≥45%), blast development rate 59.3% (≥40%), successful biopsy 98% (≥90%), and implantation rate for blastocyst 39.9% (≥ 35%) while for the 10.8% of women who had a cleavage stage transfer the implantation rate was 19.7% (≥25%).

Limitations, reasons for caution: Different laboratory protocols were used across the eight units improving the generalisability of the findings. A quality control assessment of the reproducibility of classification of a good quality blastocyst indicated substantial variability. Patients with cleavage stage embryo transfer were of poor prognosis, driving a move to blastocyst transfer for all.

Wider implications of the findings: We found that in the proposed reference population suggested within the Vienna Consensus that the competency values as defined as the mean value observed are appropriate and can be used for routine quality management of the ART laboratory.

Trial registration number: Not applicable

P-794 Chromosomal abnormalities after ICSI in relation to sperm parameters: results in 1114 fetuses and 1391 neonates from a single-center

F. Belva[1], M. Bonduelle[1], A. Buysse[1], A. Van den Bogaert[1], F. Hes[1], M. Roelants[2], G. Verheyen[3], H. Tournaye[3], K. Keymolen[1]

[1]UZ Brussel, Medical Genetics, Jette- Brussels, Belgium ;
[2]KU Leuven, Department of Public Health and Primary Care, Leuven, Belgium ;
[3]UZ Brussel, Center for Reproductive Medicine, Jette- Brussels, Belgium

Study question: Is there a relationship between karyotype abnormalities in fetuses and children conceived by intracytoplasmic sperm injection (ICSI) and their father's semen parameters?

Summary answer: *De novo* chromosomal abnormality rate in pre-and postnatal karyotypes of ICSI offspring was higher than in the general population and related to fathers' sperm parameters.

What is known already: Several studies have reported a higher rate of *de novo* chromosomal anomalies in ICSI fetuses but recent data from large cohorts are limited. Overall, reported prevalences of non-inherited karyotype aberrations are increased in fetuses conceived after ICSI and vary between 1.6% and 4.2%. Only few studies focus on the relation between karyotype anomalies in ICSI offspring and semen parameters of their fathers. Furthermore, an increased incidence of abnormal karyotypes in ICSI neonates has been described, but the rates vary widely across studies.

Study design, size, duration: We report on karyotype results from prenatal testing by means of chorionic villus sampling and amniocentesis and results from postnatal blood sampling from offspring conceived by ICSI in a single-center. Ongoing pregnancies resulting from an oocyte retrieval between January 2004 and December 2012 and after transfer of fresh ICSI embryos obtained after ejaculated or non-ejaculated sperm were included. Pregnancies following frozen embryo transfer, oocyte or sperm donation, IVF, PGT and IVM were excluded.

Participants/materials, setting, methods: From the 4816 eligible ICSI pregnancies, information on pregnancy outcome was available for 4267 pregnancies. Prenatal testing was performed in 22.3%, resulting in a diagnosis in 1114 fetuses. A postnatal karyotype was obtained in 29.4% of the pregnancies resulting in a total of 1391 neonates sampled. The prevalence of chromosomal anomalies according to maternal age and semen quality was analyzed by logistic regression. For definitions of normal semen quality, the WHO reference values were adopted.

Main results and the role of chance: An abnormal fetal karyotype was found in 41 fetuses (29 singletons, 12 multiples) (3.7%; 95%CI: 2.7-4.9%): 36 anomalies were *de novo* (3.2%; 95%CI 2.3-4.4), either numerical (n=25), sex (n=6) or structural (n=5) and 5 anomalies were inherited. Logistic regression analysis did not show a significant association between maternal age and having a *de novo* chromosomal abnormality (OR 1.05; 95%CI 0.96-1.15). In all but one case, fetuses with an abnormal karyotype were conceived after ICSI using ejaculated sperm.

Abnormal karyotypes were observed in 14 (1.0%; 95%CI: 0.6-1.7) out of 1391 postnatal samples of children born after ICSI who were not tested prenatally: 12 were *de novo* anomalies and 2 were inherited balanced karyotypes. The 14 abnormal karyotypes were all found in children born after ICSI using ejaculated sperm.

The odds of a *de novo* karyotype aberration increased with maternal age when combining pre- and postnatal data (OR 1.11; 95%CI 1.04-1.19). A higher rate of *de novo* chromosomal abnormalities was found in fetuses and children of couples with men having low (AOR 2.10; 95%CI 1.14-3.78) and extremely low sperm concentration (AOR 1.9; 95%CI 1.05-3.45) and a non-significant higher rate was found in men with below-reference sperm counts (AOR 1.70; 95%CI 0.95-3.06).

Limitations, reasons for caution: We cannot exclude that the observation of an increased prevalence of karyotype anomalies in ICSI offspring is due to enhanced surveillance after ART given the lack of a control group. Although we did not find more chromosomal anomalies after non-ejaculated sperm, the small numbers do not allow firm conclusions.

Wider implications of the findings: The observed increased risk of a *de novo* karyotype anomaly after ICSI in couples with poor sperm warrants continued counseling towards prenatal testing. The widespread use of innovative non-invasive prenatal testing will result in larger datasets, adding to a balanced estimation of the prevalence of karyotype anomalies in ICSI offspring.

Trial registration number: not applicable

P-795 Patient age does not influence all decisions during an IVF cycle according to HFEA data

C. JACQUES[1], J. Chambost[1], C.A. Pena[1], M. Kotrotsou[2], C. Hickman[3]

[1]Apricity, AI Team, Paris, France ;
[2]Apricity, Medical Team, London, United Kingdom ;
[3]Apricity, AI Team, London, United Kingdom

Study question: One of the most important factors of success for an IVF cycle is the patient's age, does it influence all steps of a cycle?

Summary answer: According to literature, age is one of the key factors in the success of IVF, but age should not influence all decisions during a cycle.

What is known already: Different factors can influence the outcome of an IVF. Several studies observed a relation between the life birth rate (LBR) and factors such as BMI, smoking, number of eggs collected, cause of infertility, etc. According to most studies, the factor having the most influence is the patient's age. In fact, we tend to quote chance of pregnancy based on age. In fact, in most studies to predict SR from a patient's profile with machine learning (ML) algorithm, the common feature is the age (Hassan, 2018, «A Machine Learning Approach for Prediction of Pregnancy Outcome Following IVF Treatment »).

Study design, size, duration: This is a retrospective study on publicly available HFEA data from 2000 to 2016 and from 2010 to 2016. Only fresh cycles with patients' own gametes were selected and divided in age cohorts.

Several moments of a cycle were studied: egg collection, cleavage/blastocyst culture, embryo transfer (single or multiple) and finally the life birth rate in relation to the patient's profile (age, infertility cause, etc.).

Participants/materials, setting, methods: About 300000 cycles from 2010-2016 (DB_2010) and about 200000 (DB_2000) additional cycles from 2000 to 2016 were used for this study and divided into age cohorts: 43% of 18-34 year-old (y.o.), 22% of 35-37 y.o., 15% of 38-39 y.o., 14% of 40-42 y.o., 4% of 43-44 y.o. and 2% of 45-50 y.o.

Multivariate statistical analyses were conducted on those data and 6 algorithms of Machine Learning such as Random Forest were learnt.

Main results and the role of chance: According to DB_2010, although the optimum number of eggs to get the maximum LBR differs by age (18 eggs collected under 35 y.o. whereas over 35 y.o., LBR increases with the number of

eggs collected), there is a strong correlation between the number of fresh eggs collected and the number of embryos created (y=0.49x, R2=0.98) regardless of the age of the patients.

Regarding day of transfer, irrespective of patient age, blastocyst transfer offers better SR than cleavage transfer. According to DB_2000, delaying embryo transfer increased LBR in a similar way from day 1 to 5 across all age groups (LBR +20% under 37 y.o. and +10% over 37 y.o.).

Age should not be considered within a single/multiple embryo transfer (SET/MET). MET provided no benefit for young patients (Odds Ratio, OR, 1.0±0.02CI) and only marginal for older patients (OR around 1.4). This benefit should be considered with the risk of multiple LB (1.4 avg. number of LB under 35 y.o. decreased to 1.1 over 45 y.o.).

In addition to overall analysis, the DB_2010 allows you to customize results. The Fertility Predictor (https://www.apricity.life/fertility-predictor) based on 6 ML models predicts the SR according to the patient's profile, age, infertility cause, etc.

Limitations, reasons for caution: This is a retrospective study based on HFEA data, hence has inherent bias. Although the data (approximately 300000 cycles) may reflect the general population in the UK, there is more data for the younger cohorts. That's why, for better results, more data is needed.

Wider implications of the findings: Using AI in studies implies the use of a large dataset to reduce bias and improve generalization of the model, and the need to create a collaborative infrastructure which allows to increase the size of training datasets.

Trial registration number: NA

P-796 Different placental vascularisation after ART?

C. Vrouwenraets[1], R. Van Golde[1], S. Al-Nasiry[1], A. Van Montfoort[1]

[1]MUMC+, Obstetrics and Gynaecology, Maastricht, The Netherlands

Study question: Is early placental vascularisation related to birthweight in naturally and ART conceived -pregnancies?

Summary answer: This study provides an indication that early placental vascularisation is different in ART-pregnancies , but this is not related to a difference in birthweight.

What is known already: It is well known that ART-children show a higher risk of adverse neonatal outcomes, such as low birthweight, compared to naturally conceived children. The underlying aetiology is still unknown, but we expect that placental vascularisation plays a major role. We hypothesize that; 1) there is an association between birthweight and placental vascularization in general and; 2) ART placental vascularisation is lower and results in a lower birthweight compared to naturally conceived pregnancies.

Study design, size, duration: This prospective pilot study was performed at the MUMC+, department of Obstetrics and Gynaecology. At 12, 16 and 20 weeks of gestation a placental ultrasound was performed measuring the placental vascularization index (VI) using 3D Power Doppler and VOCAL. Birthweight and gestational age at time of delivery were collected after delivery.

Participants/materials, setting, methods: Women aged above 18 years, who conceived naturally (NC) or via IVF with or without ICSI (ART) were included. Multiple pregnancies were excluded.

Main results and the role of chance: In total 112 subjects were included, 57 in the ART-group and 65 in the NC group. The median VI at 12 weeks of gestation was 0.51% [IQR 0.2-2.8] in the NC group and 0.17% [IQR] 0.01-1.1] in the ART group (*P*=0.492). At 16 and 20 weeks respectively the median VI was 0.25% [0.1-1.5] (NC) vs 0.42% [0.1-1.0] (ART)(*P*=0.078) and 0.4% [0.2-1.2] (NC) vs 0.12% [0.03-0.4] (ART)(*P*=0.052). No association was found between placental VI and fetal birthweight at 12 (*P*=0.46), 16 (*P*=0.58) and 20 (*P*=0.33) weeks of gestation.

Limitations, reasons for caution: The number of participants in this pilot study is limited. Secondly, the control group of NC pregnancies is recruited in a tertiary hospital, resulting in an increased number of high risk pregnancies , possibly underestimating the results.

Wider implications of the findings: The results of this pilot study suggest that placental vascularisation might differ between ART pregnancies and NC-pregnancies. Currently a prospective well-powered cohort study on this topic is carried out, in which previous limitations regarding the control group are taken into account.

Trial registration number: x

P-797 1 million cycles: what can we learn from the UK national registry (HFEA) dataset?

C. Hickman[1], C. Jacques[2], C.A. Pena[3], M. Kotrotsou[4], J. Chambost[5]

[1]Apricity, Embryology, London, United Kingdom ;
[2]Apricity, Data Analysis, Paris, France ;
[3]Apricity, Data, Paris, United Kingdom ;
[4]Apricity, Clinical, London, United Kingdom ;
[5]Apricity, Artificial Intelligence, Paris, France

Study question: What can the HFEA data registry teach the fertility sector that will improve fertility care?

Summary answer: Although trends can be identified in the HFEA registry, the type, diversity and accuracy of data collected limits incorporation of learned knowledge into clinical practice.

What is known already: The HFEA registry records all IVF cycles performed at British fertility clinics. It is recognised as amongst the best national registries worldwide and has been the source of numerous publications attempting to find patterns that may improve practice. For instance: identifying the optimal number of eggs to be collected in order to maximise the chance of pregnancy for each patient age group; comparison in outcome for different types of stimulation; or a comparison of outcome for cleavage versus blastocyst transfer; creation of fertility predictors to help manage patient's expectations; amongst many other questions we have on optimal clinical care.

Study design, size, duration: HFEA registry is a large, national, multicentre dataset allowing for cohort analysis based on patient age, number of previous cycles, type of treatment, type of infertility, cleavage/blastocyst transfer, number of embryos transferred, type of stimulation. Outcomes include number of eggs collected, number of embryos created and utilised, clinical pregnancy, live birth. There was no access to data on embryo quality, embryo imagery, degree of mosaicism, ultrasound imagery, extended patient demographic or clinical diagnostic data.

Participants/materials, setting, methods: The HFEA registry comprises 235,614 cycles from 1995 to 1999, 492,387cycles from 2000-2009, and 495,628 cycles from 2010 to 2016 were assessed. Data trends were assessed to evaluate the quality and accuracy of the data. Data from 2010 to 2016 was consequently selected for further analysis to answer clinical practice questions using multivariate analysis. This subset had an age distribution of 43,22,15,14,4,2% for the following age groups respectively: 18-34,35-37,38-39,40-42,43-44,45-50 years.

Main results and the role of chance: Live birth rate(LBR) following fresh ET is affected by the number of mature eggs collected. In patients <35 and 35-37years, range of optimal number of eggs collected was 13-19 and 14-21 respectively, with a significant reduction in LBR at lower (understimulation) and higher egg numbers (overstimulation). At increasing age, the plateau was never reached, suggesting that the more eggs the better. However, there were insufficient patients in cohort groups at increased egg numbers and increased age. With increasing age, proportion of patients categorized with unexplained infertility tripled (8%vs30%, age <35yvs45-50); male factor infertility halved(36%vs14%). Within female infertility, proportion of patients with ovulatory disorder doubled(22%vs46%), whilst tubal infertility halved(31%vs16%). These trends are likely due to inaccuracies in data entry and/or reduction in diagnostics with increasing patient age. More than half the increase in LBR in IVF in the UK may be attributed to blastocyst culture, although there was a period of learning from 2000 to 2006 where success rates for blastpcyst culture increased with time before plateauing. The benefits of increasing LBR with multipleET compared to singleET did not increase with patient age, and was not observed in patients under 35, so that age should not be considered within an eSET policy.

Limitations, reasons for caution: Despite the large dataset, when looking at specific cohorts, there were insufficient datapoints to conclude on best clinical practice. Missing data types also limited conclusions (i.e. embryo quality, OHSS, clinical/laboratory practices, patient data).

Wider implications of the findings: Due to limitations of the current structure of the HFEA registry, fertility predictor studies and calculators based on this data cannot achieve true personalised medicine. In the era of healthcare digitisation, the fertility sector should rethink how to share better quality data to fuel structured international prospective cohort studies.

Trial registration number: NA

P-798 Triple risk of severe maternal morbidity in women with twin pregnancy obtained by oocyte donation

D. Korb[1], S. Thomas[1], S. Aurélien[2], L.R. Camille[3], S. Pietro[4], G. François[3], D.T. Catherine[5]

[1]Université de Paris- Centre for Epidemiology and Statistics Sorbonne Paris Cité CRESS- Obstetrical Perinatal and Pediatric Epidemiology Research Team- EPOPé- INSERM- INRA- Paris- France, Department of Obstetrics and Gynecology- Robert Debré Hospital- A, ;
[2]Université de Paris- Centre for Epidemiology and Statistics Sorbonne Paris Cité CRESS- Obstetrical Perinatal and Pediatric Epidemiology Research Team- EPOPé- INSERM- INRA- Paris- France, Clinical Research Unit of Paris Descartes Necker Cochin- APHP- Pa, ;
[3]Université de Paris- Centre for Epidemiology and Statistics Sorbonne Paris Cité CRESS- Obstetrical Perinatal and Pediatric Epidemiology Research Team- EPOPé- INSERM- INRA- Paris- France, Maternité Port-Royal- AP-HP- APHP- Centre-Université de Paris- Pa, ;
[4]Sorbonne Paris Cité- Faculté de Médecine- Assistance Publique-Hôpitaux de Paris- Hôpital Universitaire Paris Centre- Centre Hospitalier Universitaire Cochin- Service de Gynécologie–Obstétrique II et de Médecine de la Reproduction-Université Paris Desc, , ;
[5]Université de Paris- Centre for Epidemiology and Statistics Sorbonne Paris Cité CRESS- Obstetrical Perinatal and Pediatric Epidemiology Research Team- EPOPé- INSERM- INRA- Paris- France1.Université de Paris- Centre for Epidemiology and Statistics Sorbo, ,

Study question: Is there a difference in the risk of serious maternal complications in twin pregnancies according to the mode of conception, while differentiating infertility treatments categorization?
Summary answer: The risk of serious maternal complications was increased by 50% for twin pregnancies after IVF with autologous oocyte and almost tripled after oocyte donation.
What is known already: IVF has been recognized as a risk factor of serious maternal complications in general populations of parturients and in singleton pregnancies. In twin pregnancies, this association is less documented, with contradictory results. Moreover, these findings were limited by imprecise categorization of the mode of conception and the lack of specific analyses for exploring the causal underlying mechanisms implicated.
Study design, size, duration: In this secondary analysis of the JUMODA cohort, a national prospective population-based study of twin deliveries conducted from 2014 to 2015 in 176 French hospitals, we included all women with twin pregnancies ≥22 weeks of gestation with known mode of conception (n=8823).
Participants/materials, setting, methods: We included women with known mode of conception (n= 8748). Mode of conception was studied in 5 classes: spontaneous conception (reference group), non IVF fertility treatment (NIFT), IVF with autologous oocyte (IVF-AO), ICSI with autologous oocyte (ICSI-AO) and oocyte donation (OD). We assessed the association between mode of conception and severe acute maternal morbidity (SAMM) (composite criterion) with multivariate logistic regression. Role of intermediate factors was analyzed by structural equation modelling (SEM).
Main results and the role of chance: Among the 8748 women of the study population, 5890 (67.3%) conceived spontaneously, 854 (9.8%) had NIFT, 1307 (14.9%) IVF-AO, 368 (4.2%) ICSI-AO and 329 (3.8%) OD. Overall, 538 (6.1%) developed SAMM. Overall, 538/8748 (6.1%) women developed SAMM. Women with non-spontaneous twin pregnancy had a higher risk of SAMM than those with spontaneous twin pregnancy, after adjustment for confounders (227/2858 (7.9%) compared to 311/5890 (5.3%), aOR 1.3, 95% CI [1.1,1.6]). The risk of SAMM was higher among women with IVF-AO (108/1307; 8.3%) and OD (46/329; 14.0%) compared with the reference group (respectively aOR 1.5, 95% CI [1.1,1.9] and aOR 2.7, 95% CI [1.8,4.1]), and higher after OD than after IVF-AO (aOR 1.7, 95% CI [1.1-2.6]). Conversely, the risk of SAMM in women with NIFT (53/854; 6.2%) and ICSI-AO (20/368; 5.4%) did not differ from that of the reference group (311/5890; 5.3%) (respectively aOR 1.1, 95% CI [0.8,1.5] and aOR 0.9, 95% CI [0.6,1.5]).These risk augmentations were poorly explained by the intermediate factors tested (non-severe preeclampsia, placenta praevia and planned mode of delivery).
Limitations, reasons for caution: Beyond the confounders and intermediate factors considered in our analysis, precise causes of infertility and specificities of infertility treatments may explain the differences we found in the risk of SAMM by mode of conception. However these data were not available and could not be taken into account.
Wider implications of the findings: Knowledge of the differential risk of SAMM in women with twin pregnancies according to the mode of conception may inform the discussion of clinicians and women and help optimizing the obstetrical care for women in subgroups of mode of conception at higher risk.
Trial registration number: not applicable

P-799 Failure Mode and Effects Analysis of current IVF cryostorage processes in a large HFEA licensed clinic

R. Gregoire[1], R. Lunt[1], A. Drakeley[1]

[1]Liverpool Women's Hospital, The Hewitt Fertility Centre, LIVERPOOL, United Kingdom

Study question: What is the scale of risk associated with IVF cryostorage in a UK, Human Fertilisation and Embryology Authority (HFEA) licensed clinic?
Summary answer: Despite high standards in laboratory practice and technology, 6 High, 50 medium and 14 low risks were identified.
What is known already: The occurrence of clinical incidents in IVF cryostorage (including misplaced/unidentifiable samples, exposure to variations in temperature, and tank failure) frequently highlight the need for improved risk management and improved technologies for cryopreservation and cryostorage of gametes and embryos. There is therefore a need to conduct a comprehensive Failure Mode and Effects Analysis (FMEA): a proactive method aimed at identifying real or potential incidents in order to develop strategies to mitigate the risks with cryopreservation in IVF.
Study design, size, duration: FMEA analysis consisted of mapping the cryostorage process, from cryopreserving and cryostorage to subsequent warming - a process not currently tracked by the RFID witnessing system used by the clinic. Possible sources of error were identified, and scored with a Risk Priority Number (RPN), a product of likelihood, severity and detection of incidence associated with each risk, as previously described by Rienzi et al., 2015.
Participants/materials, setting, methods: In January 2020, an FMEA analysis was carried out in a busy IVF HFEA licensed clinic with 36 years' experience, to evaluate possible cryostorage procedural risks. Four members of the multidisciplinary IVF team including clinical, embryology and andrology specialists, completed the FMEA based on 20 years of previous clinical experience performing over 30 000 fresh and frozen IVF cycles under HFEA regulation, and risk assessed cryostorage procedures against current laboratory practice and available technologies.
Main results and the role of chance: The team identified 6 current cryostorage process phases, 56 associated process steps and 70 failure modes in current cryostorage processes, among which 50 risks were given a medium RPN score (RPN15-49) and 6 risks were scored as severe RPN (RPN>50). Failure modes identified included cryopreserved samples exposed to variable temperatures during handling; label removed from straw during cryostorage; incorrect or ineligible labelling; sample misplaced in cryostorage; difficulty in maintaining an accurate inventory; vitrified samples removed from the cryostorage environment; failure to fulfil patient cryostorage consent wishes; alarm failure; wrong sample removed from storage; tank maintenance and failure; tank contamination, and potential for staff injury. RPN scores ranged from 4 to 75 for the risks identified.
Limitations, reasons for caution: FMEA framework processes were based on the clinic's current laboratory practice. Potential errors were identified from HFEA incident reports and PUBMED literature review. Incidence likelihood was scored based on personal experience. These risk levels may not be representative of clinics operating outside HFEA regulation, and/or not utilising RFID witnessing technology.
Wider implications of the findings: FMEA is effective in supporting clinics to identify process risks and to consider alternative processes for safer practice. This FMEA demonstrates that there continues to be risks associated with IVF cryostorage processes in HFEA licensed clinics, and alternative strategies should be considered to further mitigate or eliminate these risks.
Trial registration number: Not applicable

P-800 Assisted reproductive technology and abnormal placental cord insertion: a review of adverse perinatal outcomes in over 4700 placentas

H. Leftwich[1], M. Burns[1], A. Kastrinakis[1], K. Leung[1], S. Khedr[2], S. Chauhan[1], J. Yeh[1]

[1]University of Massachusetts, Obstetrics and Gynecology, Worcester, U.S.A. ; [2]University of Massachusetts, Pathology, Worcester, U.S.A.

Study question: Do pregnancies conceived by assisted reproductive technology have higher rates of poor perinatal outcomes regardless of the location of the placental cord insertion (PCI)?
Summary answer: Pregnancies conceived by assisted reproductive technology are associated with higher rates of stillbirth in cases of abnormal PCI and cesarean delivery in normal PCI cases.
What is known already: Abnormal placental cord insertion, specifically velamentous insertion, has been associated with an increase in preterm delivery, low birth weight, neonatal intensive care admissions and emergent cesarean delivery. Both marginal and velamentous are abnormal placental cord insertions and affect less than ten percent of deliveries. Assisted reproductive technology (ART) has been associated with some similar placental-mediated pregnancy complications. It is unclear whether ART has an additional impact on the rates of adverse pregnancy outcomes in this cohort of patients.
Study design, size, duration: We performed an IRB approved retrospective chart review of 4718 singleton and twin placentas sent to pathology at the University of Massachusetts between January 2011-December 2019. Women were excluded if they delivered outside our institution or had a delivery before 24 weeks gestation.
Participants/materials, setting, methods: Chart review was performed and documented in a password protected electronic database, REDCap. Placental pathology reports were reviewed and evidence of abnormal placental cord insertion, either velamentous or marginal, were recorded. Demographics and adverse perinatal outcomes were recorded. Chi-square test was used for categorical and student T-test for continuous variables. Logistic regression was used to control for potential confounders. P less than 0.05 was used for statistical significance.
Main results and the role of chance: Of the 4718 pregnancies that were reviewed, 194 were conceived via ART. There were 483 cases of abnormal placental cord insertions. Our cohort contained 616 cases of twin gestations, and 4102 singletons. Pregnancies conceived via ART were more likely older mothers in both the abnormal and normal PCI groups. After controlling for the number of fetuses, pregnancies conceived by ART with placentas with normal placental cord insertion (PCI), had a 47 percent increase in odds of cesarean delivery (OR 1.47 [1.04-2.08], p=0.03). For those with abnormal PCI, ART was associated with an increase in odds of stillbirth (OR 5.31 [1.52-18.57], p=0.01). No other adverse outcomes were different between ART versus spontaneous conception in either normal or abnormal PCI.
Our findings show higher rates of stillbirth in cases with abnormal PCI compounded by conception utilizing ART as compared to controls. While other outcomes such as Apgar <7 at 1 minute, preterm birth or small for gestational age infants were not different among our groups, this may be due to small incidence of such adverse outcomes. Our findings suggest a closer follow up may be warranted if abnormal PCI is diagnosed on prenatal ultrasound in patients who conceived using ART.**Limitations, reasons for caution:** Limitations of our study include the retrospective nature, which does introduce bias, as not all placentas are sent to pathology at our institution. Additionally, each of our adverse outcomes are rare occurrences, which may explain the lack of difference in each outcome.
Wider implications of the findings: While there is remains some controversy regarding the risk for poor perinatal outcomes regarding abnormal placental cord insertion, our study suggests more intense monitoring, such as antenatal testing, may be appropriate when abnormal PCI is detected on prenatal ultrasound in those conceived with ART. Future studies are warranted.
Trial registration number: not applicable

POSTER VIEWING SESSION
STEM CELLS

P-801 Endometrial regeneration with establishment of improved culture methods for endometrial epithelial cells: co-culture with feeder cells.

R. Yokomizo[1,2,3], Y. Fujiki[1], H. Kishi[2], H. Sago[3], A. Okamoto[2]
[1]National Center for Child Health and Development, Department of Reproductive Biology- Center for Regenerative Medicine, Tokyo, Japan ; [2]The Jikei University School of Medicine, Department of Obstetrics and Gynecology, Tokyo, Japan ; [3]National Center for Child Health and Development, Center for Maternal-Fetal-Neonatal and Reproductive Medicine, Tokyo, Japan

Study question: Endometrial epithelial cells have limited expansion potential and it should be overcome to make regenerative medicine as a therapeutic strategy for refractory thin endometrium.
Summary answer: We successfully cultured endometrial epithelial cells until passage 5 for two and a half months using human embryonic stem cell-derived feeder cells.
What is known already: Various therapeutic approaches including hormone replacement to overcome thin endometrium, however, they remain a challenge in reproductive medicine with only slight enhancements attained with the currently available treatment. Regenerative medicines based on cellular approaches have the potential to provide new therapeutic methods, however, endometrial epithelial cells have limited expansion potential and it should be overcome to make regenerative medicine as an available therapeutic strategy.
Study design, size, duration: Endometrial samples from four healthy women undergoing benign gynecological surgery without any endometrial pathology and/or exposure for exogenous hormone therapy at a university hospital were included and analyzed for *in vitro* experiments. The protocol of the present study was approved by Institutional Review Board of the National Center for Child Health and Development of Japan (approval number: 2289) and The Jikei University School of Medicine (approval number: 28-083(8326)).
Participants/materials, setting, methods: We prepared primary human endometrial epithelial cells and endometrial stromal fibroblasts from the endometrial samples by enzymatic digestion, then isolated and cultured endometrial epithelial cells with growth media on feeder cells including mouse embryonic fibroblasts, endometrial stromal fibroblasts, and human embryonic stem cell-derived feeder cells to explore the conditions applicable for regenerative medicine. We performed cell proliferation assay and investigated cellular morphology and characteristics with immunohistochemistry.
Main results and the role of chance: Co-cultivation of the endometrial epithelial cells with the feeder cells was significantly efficient compared with cultivation in the absence of the feeder cells in viewpoints of cell growth; we could not culture the endometrial epithelial cells without the feeder cells beyond passage 1 as previously reported. Among the feeder cells, mouse embryonic fibroblasts resulted in the best feeder activity for proliferation of the endometrial epithelial cells. Likewise, endometrial stromal fibroblasts showed the equivalent potential with mouse embryonic fibroblasts; we could passage the endometrial epithelial cells on these feeder cells up to passage 5 for two and a half months. Aiming to establish the culture method with easily available human cell resources, we examined the potential of human embryonic stem cell-derived feeder cells. We found that the endometrial epithelial cells were cultured on the human embryonic stem cell-derived feeder cells up to passage 5 for two and half months, which might be regarded as an alternative culture method. Furthermore, we explored protein expression of an epithelial cell-specific marker, pan-cytokeratin, and found that expression of pan-cytokeratin was preserved in the endometrial epithelial cells with serial cultivation on the feeder cells.
Limitations, reasons for caution: We need to address further a clinical model for human application.
Wider implications of the findings: Autologous transplantation of *in vitro* cultured endometrium can be a potential option for treating women with thin endometrium. Furthermore, findings from this research can lead to establishment of *in vitro* three-dimensional endometrium model and it can also be utilized to elucidate underlying mechanisms of embryo implantation.
Trial registration number: not applicable

P-802 Epithelial-mesenchymal plasticity in neonatal spermatogonial stem cell revealed by RNA-Seq and single cell ATAC-Seq

H.C. Suen[1], J. Liao[1], C.S.A. Luk[1], W.T. Lee[1], J.K.W. Ng[1], T.L. Lee[1]
[1]The Chinese University of Hong Kong, School of Biomedical Sciences, Hong Kong, Hong Kong

Study question: Whether epithelial-mesenchymal transition (EMT) occurs in spermatogonial stem cells (SSCs) and the role of EMT in SSC self-renewal and development remain unknown.
Summary answer: SSCs demonstrated epithelial-mesenchymal plasticity, as we observed EMT-like process in SSC and recapitulated EMT in vitro, and showed that EMT is involved in SSC migration.
What is known already: EMT is a biological process which epithelial cells lose cell-cell adhesion and gain cell-matrix adhesion to become mesenchymal cells. EMT participates in embryonic development, involving in processes such as organ formation and gastrulation. The reverse of EMT is mesenchymal-epithelial transition (MET), which is crucial in stem cell self-renewal. SSCs are male germline stem cells for spermatogenesis. SSC subtypes display distinct epithelial-like or mesenchymal-like gene expression signature. During postnatal development, SSCs migrate from the center of testicular cord to the basement membrane, leading to the establishment of SSC pool. Nonetheless, the cellular mechanisms involved in SSC migration remain elusive.
Study design, size, duration: We hypothesize that, EMT facilitates cell migration of SSC, while MET maintains the undifferentiated stem cell population. To recapitulate the EMT process in SSCs in vitro, we devised a novel SSC culture method through controlling extracellular matrix substrate. We then defined the transcriptional and epigenetic program by RNA-Seq and single cell ATAC-Seq (scATAC-seq). Lastly, we performed an in vitro assay by applying small molecules to evaluate the effects of candidate regulators towards cell migration.
Participants/materials, setting, methods: Oct4+/Kit- SSCs were isolated from PND6 mouse testes for culture. SSCs were cultured on Matrigel of different concentration, which high (5-20 μg/cm2) and low concentration (0.1-0.5 μg/cm2) of Matrigel promoted formation of flat-shaped colonies (fSSCs) and domed-shaped colonies (dSSCs) respectively. dSSCs and fSSCs were characterized by cellular and molecular assays (FACS, RT-PCR, invasion assay, etc). dSSCs and fSSCs were also subjected to bulk RNA-Seq, bulk ATAC-Seq and scATAC-Seq for transcriptome and chromatin accessibility analysis.
Main results and the role of chance: In Oct4+/Kit- SSC culture, differentiation-primed subpopulation (Oct4-GFP-high) showed higher expression of genes related to ECM components and organization, implying ECM regulates SSC cell fate and maintenance. To investigate how ECM regulates SSCs at physical and molecular level, SSCs were cultured on different concentration of Matrigel, which promoted formation of two morphologically distinct cell populations, fSSCs and dSSCs. Gene expression analysis revealed dSSCs resemble an epithelial-like phenotype, characterized by higher epithelial marker (CDH1, Occludin) and self-renewal gene expression. In contrast, fSSCs is more mesenchymal-like and showed higher EMT-inducing TFs (TWIST, SNAIL, ZEB) and mesenchymal marker (Vimentin, N-cadherin, MMPs) expression. Concordantly, fSSCs displayed a significantly higher invasion rate.
To define the epigenetic program executed by SSCs undergoing EMT, we first performed bulk ATAC-Seq and confirmed the EMT-associated genes underwent increased chromatin openness. We then performed scATAC-Seq to reconstruct the regulatory dynamics of EMT transition and further identified epithelial, mesenchymal clusters and a cluster of partial EMT. The result not only recapitulated known EMT factors (Tgfb1, Zeb1) but also identified several potential regulators such as Sp1, Mef2d and Vdr. Lastly, we further demonstrated TGF-β signaling pathway plays in EMT of SSC in vitro by applying TGFBR1 inhibitor, which substantially inhibited SSC migration.
Limitations, reasons for caution: In this study, we have demonstrated EMT process in SSC in vitro. However, the link between this phenomenon and in vivo postnatal SSC development is yet to be completely elucidated. Therefore, the role of EMT in vivo warrants further investigation.
Wider implications of the findings: This study implied that EMT, or EMT-like process, can occur in SSC and modulate its migratory ability. Our results shed light on the novel molecular mechanisms underlying postnatal spermatogonial development and give insight into the clinical application of SSC transplantation to restore fertility after cancer treatment at adolescence.
Trial registration number: not applicable

P-803 Histone modifications facilitate the specification of human primordial germ cells from induced pluripotent stem cells

S. Yang[1], S. Peng[2], S. Ding[3], H. Xu[1], Y. Cao[1], Y. Huang[1]

[1]Shenzhen University General Hospital- Shenzhen University Clinical Medical Academy, Department of Obstetrics and Gynecology, Shengzhen, China ; [2]Central South University, the Key Laboratory of Carcinogenesis of The Chinese Ministry of Health and Cancer Research Institute, Changsha, China ; [3]The University of Hong Kong-Shenzhen Hospital, The Center of Reproduction Medicine, Shenzhen, China

Study question: Can histone modifications facilitate the induction of human primordial germ cells (PGCs) from induced pluripotent stem cells (iPSCs) *in vitro?*
Summary answer: Histone deacetylase inhibitors (HDACi) panobinostat promoted the differentiation of human PGCs from iPSCs *in vitro.* The induced PGCs showed high levels of histone acetylation status.
What is known already: Histone modifications modulate DNA accessibility and chromatin structure, and further affect gene expression and cell differentiation. It has also been confirmed that human iPSCs can differentiate into PGCs. But we did not know the histone status of differentiated PGCs from iPSCs *in vitro*, and the relationship of histone deacetylation and the induced differentiation of PGCs from iPSCs *in vitro*.
Study design, size, duration: VASA-GFP iPSCs were used for induction differentiation experiments. The induction efficiency of PGCs from VASA-GFP iPSCs were compared between two groups, which one is induction medium adding the HDACi (panobinostat) at day 4 and other is no adding HDACi. During inducted differentiation, the histone status was analysed at day 5 and day 8.
Participants/materials, setting, methods: The HDACi panobinostat is added after pre-induction for deacetylation at day 5. The final concentration was 8 nM. These cells were cultured three days with HDACi panobinostat and PGC medium for downstream experiments. We analyzed the histone acetylation dynamics in human PGCs at day 5 and day 8 by ChIP-sequencing and the efficiency of induced differentiation to ask whether the histone deacetylation accelerated the differentiation of human PGCs from iPSCs.
Main results and the role of chance: Our results showed in our experiments, we added histone deacetylase inhibitors panobinostat during the induction differentiation of PGCs from human iPSCs. Our results showed the human PGCs derived from iPSCs displayed high levels of histone acetylation marks in H3K9ac and H3K27ac, and also showed, in HDACi panobinostat group, VASA-GFP positive cells were 23.5 ± 4.65%, 48.1±5.65% on day 5 and day 8 induction, but in no HDACi panobinostat group, VASA-GFP positive cells were 18.6 ± 3.15%, 41.4 ± 3.48% on day 5 and day 8 induction, indicating histone modifications facilitated the specification of human primordial germ cells from pluripotent stem cells during induction differentiation *in vitro.*
Limitations, reasons for caution: In this study, the global epigenetic reprogramming process during induction differentiation of PGCs from iPSCs *in vitro* remains to be determined.
Wider implications of the findings: This provides a new and high-efficiency way for PGC induction differentiation from iPSCs *in vitro*.
Trial registration number: 'not applicable

P-804 Feasibility of parental genome cloning

P. Xie[1], A. Trout[1], R. Setton[1], Z. Rosenwaks[1], G. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: We tested the feasibility of generating biparental conceptuses through the fusion of haploid male and female pseudo-blastomeres.
Summary answer: Biparental complementation was achievable through fusing a single 2-cell–stage male pseudo-blastomere with the female counterpart; this post-syngamy reconstruction achieved full preimplantation development.
What is known already: Male haploid embryos can be generated through sperm injection of enucleated oocytes, while the maternal counterpart can be achieved by activating metaphase II oocytes by artificially inducing calcium oscillations. These haploid embryos can be used to clone scarce gametes for reproductive uses in both genders as well as for heterozygosity identification and eventual heritable genomic editing at the pre-syngamy level. Dispermic and digenic embryos can be obtained through nuclear transfer; however, these embryos do not achieve normal preimplantation development because of gender-related imprinting.
Study design, size, duration: A single cohort of mouse metaphase II oocytes was divided into groups: 1) generating male pseudo-blastomeres by inseminating

enucleated oocytes, 2) activating oocytes to yield female pseudo-blastomeres, and 3) generating control ICSI conceptuses. Biparental reconstruction was performed by subzonal pseudo-blastomere replacement of haploid male and female embryos at the 2-cell stage. Constructs were cultured for 96h to obtain full preimplantation development and compared with control. Cleavage rates were compared using t-test with 0.05 considered significant.

Participants/materials, setting, methods: To generate male pseudo-blastomeres, metaphase II oocytes from B6D2F1 mice were treated with cytochalasin B, enucleated by herniating spindle region under Oosight™ visualization, injected with a spermatozoon from same strain and cultured up to 2-cell stage. Female counterparts were generated by parthenogenesis using calcium ionophore and allowed to reach first division. At 2-cell stage, a female pseudo-blastomere was replaced by a male counterpart and fused by Sendai virus. Control embryos were generated by piezo-actuated ICSI.

Main results and the role of chance: A total of 60 oocytes were enucleated, and 57 ooplasts were obtained. Out of 57 ooplasts injected with sperm heads, 43 survived (74%), and all developed a single male pronucleus 4-6h post-ICSI. After culturing for approximately 20h, 40 androgenic embryos entered first zygotic division (93%). For the female counterparts, 52 out of 60 oocytes (86.7%) extruded the second polar body and formed a single female pronucleus. After approximately 20h, 50 2-cell haploid female embryos were obtained. Forty reciprocal pseudo-blastomere transfers were performed with a fusion rate of 90%. The cleavage rates of reconstructed conceptuses into 2-cell embryos (77.8%) and 4-cell embryos (69.4%) were comparable to the control group at 86.7% and 83.3%, respectively. However, morula compaction (50.0%) and blastocyst development (44.4%) of reconstructed conceptuses were significantly lower than the control ICSI conceptuses, with 83.3% morula compaction and 83.3% blastocyst formation rates ($P < 0.001$ and $P < 0.01$, respectively). Full preimplantation development was achieved in only 44.4% of the constructs; the remainder exhibited abnormal rotational holoblastic cleavage with a high level of fragmentation.

Limitations, reasons for caution: Although both paternal and maternal gamete cloning was achievable, the embryo development potential appears to deteriorate prior to embryo compaction. While ooplasmic heteroplasmy was prevented, reconstructed embryos may have premature loss of imprinting and abnormal syngamy resulting in aneuploidy.

Wider implications of the findings: This technique offers the possibility of propagating male and female genomes for reproductive or diagnostic applications. These cloned gametes can be utilized synchronously to reconstitute biparental conceptuses. Sibling blastomeres could be propagated, cryopreserved, or genetically screened and processed for eventual genomic editing.

Trial registration number: not applicable

P-805 Investigating the potential use of autologous platelet-rich plasma (PRP) intra-ovarian infusion treatment for ovarian rejuvenation in menopausal and peri-menopausal women: a prospective pilot study

K. Pantos[1], M. Simopoulou[2], A. Pantou[1], S. Grigoriadis[2], P. Tsioulou[2], E. Maziotis[2], N. Nitsos[1], A. Rapani[2], P. Giannelou[2], G. Kokkali[1], M. Koutsilieris[2], K. Sfakianoudis[1]

[1]Genesis, Medical, Chalandri- Athens, Greece ;
[2]National and Kapodistrian University of Athens- School of Medicine, Physiology, Athens, Greece

Study question: Could autologous PRP intra-ovarian infusion restore ovarian function promoting menstrual cycle and hormonal profile regulation and enable reproductive potential in menopausal and peri-menopausal women?

Summary answer: Intra-ovarian PRP infusion may restore ovarian function enabling folliculogenesis, and may promote menstrual cycle regulation enabling a reproductive dynamic for menopausal and peri-menopausal women.

What is known already: Women of advanced age presenting with menopausal symptoms may opt for hormone replacement (HR). However, HR is presenting with limitations. In order to address infertility, oocyte donation is employed in the context of ART. Nowadays, women of advanced age investigate alternative options in their quest to ensure a pregnancy employing their own oocytes. Recently published studies demonstrate that PRP intra-ovarian infusion may restore ovarian function in this population. However, provided data lucks robustness. This is the first pilot study aiming to provide sufficient data regarding PRP application for ovarian rejuvenation in menopausal and peri-menopausal women.

Study design, size, duration: This prospective pilot study was carried out between 1 February, 2017 and 31 January, 2019 at the Centre of Human Reproduction, Genesis Athens Clinic, Athens, Greece. Participants were included in the study following oral and written consent. Study's participants were divided in two cohorts according to the menopausal symptoms reported namely, peri-menopausal and menopausal groups. Thirty women were included in each group. In total, 60 participants were recruited to participate in the study.

Participants/materials, setting, methods: Participants were ≥40 years old presenting with menstrual cycle irregularities (peri-menopausal) or amenorrhea for at least 12 months (menopausal). Prior to PRP treatment, basic investigation, including Antral Follicle Count (AFC), Anti-Mullerian Hormone (AMH), gonadotropin and estradiol assessment, was performed. Subsequently, participants were subjected to PRP infusion. The follow-up period was three months. Regarding both of groups, the primary outcomes were: progressive reduction of Follicle Stimulating Hormone (FSH) levels and menstrual cycle restoration.

Main results and the role of chance: Regarding the peri-menopausal group, 24 women (80%) achieved menstrual cycle regulation. Mean age was 43.25±1.42 years. All 24 patients presented with at least three consecutive regular menstrual cycles following PRP. A progressive reduction was observed regarding FSH levels, reaching lowest levels in the third menstrual cycle following PRP (18.51±2.62 vs 15.28±4.03, p-value=0.021). From the first menstrual cycle following PRP, an increase was observed regarding AMH levels (0.96±0.28 vs 1.42±0.16, p-value<0.001), estradiol levels (29.67±3.82 vs 39.50±2.06, p-value<0.001) and AFC number (1.54±0.51 vs 2.79 ± 0.78, p-value<0.001). Four patients achieved natural conceptions. Regarding the menopausal group, 13 women (43.3%) achieved menstrual cycle restoration. The mean age was 48.85±1.57 years. All 13 patients presented with at least three consecutive regular menstrual cycles following PRP. The mean time for menstrual cycle recovery was 40.92±7.57 days. A progressive reduction was observed regarding FSH levels, reaching the lowest levels in the third menstrual cycle following PRP compare to baseline (80.27±5.03 vs 30.55±2.50, p-value<0.001). From the first menstrual cycle following PRP, an increase was observed in regards to AMH levels (0.13±0.03 vs 0.32±0.08, p-value<0.001), estradiol levels (14.01 ± 2.59 vs 22.19±5.63, p-value<0.001) and AFC number (0 vs 1.31± 0.48, p-value<0.001). One patient achieved natural conception.

Limitations, reasons for caution: The absence of control groups, mainly attributed to the observational nature of this pilot study, consists of the most significant limitation of the study. Furthermore, the small sample size could serve as an additional limitation. The relatively small follow-up period could serve as another reason for caution.

Wider implications of the findings: Autologous intra-ovarian PRP infusion may restore ovarian function, folliculogenesis reactivation, recovery of menstrual cycle, and enhancement of the hormonal profile regarding peri-menopausal and menopausal women. This may provide the basis for enabling a reproductive dynamic. Future studies are required to provide robust evidence in regards to PRP efficiency and safety.

Trial registration number: Not applicable

P-806 Similar population of small CD133+ stem cells from recurrent ovarian and testicular cancer expressing some markers of germline (DDX4, PRDM14) and pluripotency (SSEA4)

I. Virant - Klun[1], V. Salamun[1], N. Kenda - Suster[1], S. Novakovic[2], P. Skerl[2], S. Smrkolj[1]

[1]University Medical Centre Ljubljana, Department of Obstetrics and Gynaecology, Ljubljana, Slovenia ;
[2]Institute of Oncology Ljubljana, Molecular Diagnostics, Ljubljana, Slovenia

Study question: Are there stem cells that persist from the embryonic period of life (indifferent gonads) and are associated with both ovarian and testicular cancer ?

Summary answer: There is a population of CD133+ stem cells expressing some markers of germline and pluripotency which are associated with both ovarian and testicular cancer.

What is known already: Numerous publications indicate that ovarian cancer is associated with stem cells that are involved in cancer, metastasis, and chemoresistance, but these cells express different phenotypes/markers; there is no

generally accepted population of ovarian cancer-related stem cells. Testicular cancer has been less studied in terms of cancer-related stem cells. Spermatogonia are well-researched and of clinical importance (e.g., freezing of spermatogonia for fertility preservation), but testicular cancer is more attributed to their pre-stage – unknown gonocytes. Therefore, the purpose of this study was to identify stem cells that are common to both ovarian and testicular cancer and express germline and pluripotency-related markers.

Study design, size, duration: During three years, ascites was retrieved from 10 patients with recurrent serous ovarian cancer and testicular tissue from 4 patients with bilateral seminoma testicular cancer. Cell culture was established from each sample and CD133+ cells were sorted by magnetic-activated cell sorting (MACS). Sorted cells were proliferated (FSH+valproic acid), analyzed for genes/markers of germinal lineage (DDX4, PRDM14) and pluripotency (SSEA4), and differentiated into other types of cells to compare cells from ovarian and testicular cancer.

Participants/materials, setting, methods: Ascites was obtained under sterile conditions, centrifuged and cultured in RPMI medium supplemented with insulin, EG, bFGF, FBS, and penicillin/streptomycin. Testicular tissue was enzymatically degraded with collagenase and cultured in supplemented DMEM-F12 medium. After MACS-sorting (Miltenyi), CD133+ cells were proliferated (FSH+valproic acid), analyzed with qPCR and immunocytochemistry, and differentiated into adipogenic, osteogenic and neural-like lineages; adipogenic cells were confirmed by Oil Red O, osteogenic cells with Von Kossa staining, and neural-like cells with S100 staining.

Main results and the role of chance: In both ovarian and testicular cancer, there was a similar population of small (diameters of up to 5 .µm) and yellow shining CD133+ cells after MACS. In both types of cell cultures, these cells were more abundant than in normal, non-malignant ovarian and testicular tissue. Ascites cells expressed *CD133* and *DDX4* genes at a lower level than testicular cancer cells (6.14±1.1 and 9,55±2.15 vs. 20.85±5.40 and 763.99±186.05) at a comparable cell count; they did not express the primordial germ cell-related *PRDM14* gene, while the testicular cells did. Both, ascites and testicular cancer cells expressed CD133, DDX4, PRDM14, and SSEA4 markers after immunocytochemistry. After MACS, all cells were dormant but proliferated strongly after 2-3 months of exposure to FSH+valproic acid. The proportion of cells was differentiated into adipose, osteogenic, and neural-like cells in appropriate differentiation media. It can be concluded that there is a similar CD133+ stem cell population in both ovarian and testicular cancer cell cultures expressing stem cell and germline markers, and can differentiate into other types of cells. Nevertheless, there are also differences at the molecular level. These small cells are suggested as stem cells – gonocytes/VSELs that persist in gonadal tissues from the embryonic period of life.

Limitations, reasons for caution: Further characterization of discovered cells is required, including transplantation into SCID mice for testing the formation of tumors.

Wider implications of the findings: This new knowledge may lead to a better understanding and treatment of ovarian/testicular cancer in the future.

Trial registration number: Republic of Slovenia Medical Ethical Committee approval 154/07/10

P-807 Ovarian Autologous Platelet rich plasma therapy in ovarian insufficiency: An alternative for donor oocyte

R. Sahay[1], S. Shukla[1]

[1]India IVF Clinic, Infertility, Delhi, India

Study question: Does intraovarian infusion of autologous platelet rich plasma increases the egg/embryo quality ?

Summary answer: Intraovarian infusion of autologous platelet rich plasma represents a new effective and safe alternative method for infertile patients with low ovarian reserve .

What is known already: The major function of platelets is to prevent acute blood loss and repair vascular walls and adjacent tissues , they also activate and aggregate to release granules containing growth factors, such as Transforming Growth Factor -β, Platelet Derived Growth Factor , Insulin like growth factor , Vascular Endothelial Growth Factor, Epidermal Growth Factor and Fibroblast Growth Factor -2 , which stimulate the inflammatory cascade and healing process. It is fractionated plasma from autologous blood and contains concentrated platelets. It is nowadays widely applied in various clinical scenarios, in orthopedics, ophthalmology, oromaxillary and wound healing to improve the tissue regeneration.

Study design, size, duration: Case series study ,

Seven patients were recruited ,

Duration is of six months (August 2019-January 2020)

Participants/materials, setting, methods: One IVF cycle canceled for poor follicular recruitment , Anti mullerian hormone < 1 ng/ml , Follicle stimulating hormone >16mIU/mL . At tertiary level IVF centre after taking written consent 15 ml of venous blood was drawn from Arthrex Double syringe system , centrifuged by ACP centrifuge system for 10 min. The supernatant pellet 3ml was infused bilaterally intraovarian by single lumen needle under transvaginal ultrasound guidance with multiple punctures under anesthesia.

Main results and the role of chance: In this study, seven patients had extraordinarily poor ovarian response and the ovaries were non-responsive to conventional controlled ovarian stimulation, resulting in cycle cancellation, need for donor eggs, low possibility of pregnancy and heavily emotional distress.

After application of intraovarian autologous platelet rich plasma , the ovarian response was satisfactory (FSH level decreased by 40% , AMH level increased by 10% , serum estradiol levels increased by 35% ,healthy oocytes were retrieved and blastocyst were made) in five of the patients, and in rest two we are planning repeat cycles as the response was inadequate . They all showed features of restoration of menstruation and symptomatic changes in their body.

Our findings suggest that it is able to increase the ovarian function and help the patients make embryo from there own eggs .

Since PRP is prepared from autologous blood, theoretically there are minimal risks for disease transmission, immunogenic reactions. No infection or injury was observed.

As the diagnosis of IVF failure is always tentative, clinical efficacy and reproductive outcome of Platelet rich Plasma in this indication is difficult to assess and predict.

Limitations, reasons for caution: Various extensive research have been done regarding optimal treatment for controlled ovarian stimulation . However, till date no such approach is there which gives guaranteed results .

Wider implications of the findings: The findings provide evidence for future randomized, controlled trials with large sample size in this field.

Additional research is needed to clarify (and enhance) which PRP components are responsible for altered ovarian function, and to identify predictive characteristics for patients most likely to benefit from this intervention.

Trial registration number: not applicable

P-808 Somatic cell nuclear transfer of different diploid cells into an enucleated oocyte aimed at nuclear haploidization

A. Trout[1], P. Xie[1], R. Setton[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: We evaluated the efficiency of oocyte-mediated haploidization with different types of donor nuclei and observed their complete preimplantation development.

Summary answer: Despite cumulus cells having a superb haploidization rate, embryo development rates were comparable between all donor cell types.

What is known already: Success with human reproductive technology is universally limited by oocyte aging, characterized by higher aneuploidy. Numerous attempts have been made to generate oocytes through the haploidization of a somatic nucleus. A large array of cell types, at different cell-cycle stages, have been proposed as candidate donor cells. Studies in primate models found fibroblasts to be superior donors than cumulus cells, while others found mouse embryonic stem cells to be more effective than differentiated cells. Nonetheless, in the most recent study, cumulus cells have been used to analyze correct chromosome number and the developmental potential of artificially derived oocytes.

Study design, size, duration: Metaphase II oocytes from B6D2F1 mice were split into 4 groups: 1) to generate ooplasts for somatic cell haploidization of corona/cumulus cells, 2) embryonic stem cells, 3) fibroblasts, and 4) to generate ICSI conceptuses serving as control. Successfully haploidized oocytes were then observed for meiotic spindle development and then ICSI inseminated. Rates of successful haploidization, fertilization, and blastocyst development were compared among the different donor somatic cells. The chi-squared test was used to compare results.

Participants/materials, setting, methods: To generate ooplasts for haploidization, MII oocytes were exposed to cytochalasin B and enucleated by herniating spindle complexes under Oosight™ visualization. Afterwards, a single cell, cumulus, mES, or fibroblast was inserted into the perivitelline space of the ooplast, and fused using Sendai virus. Successful oocyte fusion and somatic cell reprogramming was verified by spindle development and extrusion of a polar body prior to injection by piezo-ICSI. Intact oocytes, solely injected by piezo-ICSI, served as control.
Main results and the role of chance: Of 338 enucleated oocytes, cumulus cells were transferred in 232, while mESCs were transferred into 49, and fibroblasts into 57, ooplasts. Following spindle development and pseudo polar body extrusion, oocytes injected with cumulus cells were found to have a significantly higher rate of haploidization (55%, $P<0.00001$), while mESC and fibroblasts had rates of 31% and 17%, respectively. The rate of fertilization, measured by the presence of two clear pronuclei and one distinct second polar body, at 4-6 hours post-insemination, was comparable among the cumulus (32%), mESC (42%), and fibroblast (29%) cohorts. However, the rate of fertilization for the control group (73%) was significantly greater than each of the donor cell groups ($P< 0.00001$). In the experimental groups, after fertilization, blastocyst development for cumulus cells (21%) was also comparable to mESCs (9%) and fibroblasts (17%). Despite no significant differences among each experimental category, when compared to the control (87%), blastocyst development was present but significantly compromised ($P<0.0001$).
Limitations, reasons for caution: This is a preliminary study on the efficiency of female genome haploidization. While this study only assessed preimplantation development, the next step would be to enhance the efficiency of embryo development and obtain live pups.
Wider implications of the findings: Once this technique is optimized, and successful post-implantation development is confirmed, it may serve to generate genotyped gametes. Implementation of somatic cell haploidization may be able to alleviate age-related female infertility by generating competent oocytes in women with premature ovarian insufficiency.
Trial registration number: N/A

P-809 Revealing the aging effect in menstrual blood-derived stem cells using CD146+ phenotype

W.T. Lee[1], J. Liao[1], C.S. Luk[1], H.C. Suen[1], J.K.W. Ng[1], T.T.H. Chan[1], M.Y. Cheung[1], M.P. Zhao[2], D.Y.L. Chan[2], W.Y. Chan[1], T.Y. Leung[2], K.L. Chow[3], Q. Lian[4], T.C. Li[2], T.L. Lee[1]

[1]The Chinese University of Hong Kong, School of Biomedical Sciences, Hong Kong, Hong Kong ;
[2]The Chinese University of Hong Kong, Department of Obstetrics and Gynaecology, Hong Kong, Hong Kong ;
[3]The Hong Kong University of Science and Technology, Department of Chemical and Biological Engineering, Hong Kong, Hong Kong ;
[4]The University of Hong Kong, Li Ka Shing Faculty of Medicine, Hong Kong, Hong Kong

Study question: Can we restore the quality of menstrual blood-derived stem cells (MenSCs) from women of advanced maternal age (AMA) for autologous cell-based therapies?
Summary answer: Selection of CD146$^+$ MenSCs could be feasible to establish personal MenSC banks with high therapeutic potential for women of AMA.
What is known already: In recent years, MenSC has become a promising strategy in regenerative and therapeutic application. It includes ovarian-related diseases, endometriosis and Asherman's syndrome (AS). MenSCs are abundant, easily and non-invasively isolated. They have high proliferative capacity and multilineage differentiation potency. Previous studies revealed that they express most mesenchymal stem cell (MSC) specific markers. Therefore, they are considered as a source of MSCs. It is well-established that there was an age-related decline in the number of CD146$^+$ in bone marrow-derived MSCs which contributed to lower therapeutic potential. However, the effects of aging on biological characteristics and gene expression of MenSC remain unclear.
Study design, size, duration: Menstrual blood blood from young (age ≤ 26 years) and elderly (age > 35 years) donors were freshly collected. Both *in vivo* and *in vitro* MenSCs were extracted for investigating the aging effects.
Participants/materials, setting, methods: Menstrual blood mononuclear cells (MMCs) were isolated using Ficoll-Hypaque density-gradient centrifugation. MMCs were directly analyzed by standard cell surface marker (CD90, CD105 and CD146) using fluorescence-activated cell sorting (FACS). Meanwhile, MenSCs collected from the two age groups were cultured for further evaluation including morphology, adenosine triphosphate (ATP) content and immunophenotypes.
Main results and the role of chance: Subpopulation of individual immunophenotype (CD90$^+$/CD105$^+$/CD146$^+$) in *in vivo* MMC population differs among donors as the number of cells detached from endometrium varies in each collection. Meanwhile, we revealed that the percentage of MenSCs declined dramatically in *in vivo* MMC population when the storage time of menstrual blood increased. Therefore, fresh samples were essential in order to collect larger number of *in vivo* MenSCs. There was no obvious aging effect on the number of *in vivo* MMCs and subpopulations of individual immunophenotypes. Surprisingly, we discovered that the number of CD146$^+$ MenSCs in *in vivo* MenSC population from young donors was greater than that of elderly donors by approximately 1.5-fold. Furthermore, *in vitro* MenSCs from elderly donors showed lower ATP content and expressions of MSC markers.
Limitations, reasons for caution: The number of donors in the elderly group was limited, a larger sample size is required for a confident conclusion.
Wider implications of the findings: Our study provides promising information for the potential of using CD146$^+$ phenotype to predict the age of MenSCs and the efficacy of autologous MSCs for treating elderly patients.
Trial registration number: not applicable

P-810 Future stem cell therapies in fertility preservation of prepuberal children with cancer or genetic syndromes. Improvements in the characterization of human Spermatogonial Stem Cells.

M. Myriam[1], E. Armendariz[1], L. Herrera[1], C. Rodriguez[1], N. Roig[2], L. Garcia-Quevedo[2], F. Vidal[2], M. Diaz-Nuñez[3], M.B. Prieto[4], R. Matorras[4], R. Lopez-Almaraz[5], E. Solorzano[6], S.M. Chuva de Sousa Lopes[7], A. Van Pelt[8], C. Eguizabal[1]

[1]Biocruces Bizkaia Health Research Center- Basque Centre for Blood Transfusion and Human Tissues- Barrio Labeaga s/n- Galdakao 48960- Spain., Cell Therapy- Stem Cells and Tissues Group, Galdakao, Spain ;
[2]Universitat Autònoma de Barcelona, Cell Biology Unit- Department of Cell Biology- Physiology and Immunology, Bellaterra Cerdanyola del Vallès, Spain ;
[3]Biocruces Bizkaia Health Research Center-Cruces University Hospital, Human Reproduction Unit, Barakaldo, Spain ;
[4]Biocruces Bizkaia Health Research Center-Cruces University Hospital-Basque Country University-IVI Bilbao, Human Reproduction Unit, Barakaldo, Spain ;
[5]Biocruces Bizkaia Health Research Center-Cruces University Hospital, Pediatric Department, Barakaldo, Spain ;
[6]Biocruces Bizkaia Health Research Center-Cruces University Hospital, Pediatric Surgery Department, Barakaldo, Spain ;
[7]Leiden University Medical Center, Department of Anatomy and Embryology, Leiden, The Netherlands ;
[8]Research Institute Reproduction and Development- University of Amsterdam-, Center for Reproductive Medicine, Amsterdam, The Netherlands

Study question: Is possible to improve the *in vitro* expansion and to determine the ploidy of Spermatogonial Stem Cells (SSCs) of Klinefelter Syndrome (KS) prepuberal patients?
Summary answer: We achieved an *in vitro* expansion improvement of SSCs with PRP and we determine the correct ploidy of SSCs in KS patients by DNA FISH.
What is known already: Oncologic prepuberal children after cancer treatments and prepuberal children with genetic syndromes, such as Klinefelter Syndrome, can suffer fertility problems in adulthood. The aim of this experimental fertility program is to preserve testicular tissue (with SSCs) to expand *in vitro* these stem cells for future autologous transplantation of the tissue or expanded SSCs in the adulthood. On one hand, still is needed a good SSCs expansion protocol for future spermatogenesis restoration in these patients. On the other hand, a correct ploidy determination (mosaic/pure) in SSCs of prepuberal SK patients is needed.
Study design, size, duration: We collected and processed testicular biopsies of both adults and children (oncologic and KS). Each biopsy is divided into 3 fragments: for histological study, for clinical use and for research.

Participants/materials, setting, methods: Testicular biopsy fragment for histological studies, we performed immunofluorescence to determine expression of germ cells (VASA) and SSCs (MAGEA4) markers.

Testicular biopsy fragment for clinical use is preserved in a specific cryopreservation medium with quality controls.

Testicular biopsy for research is processed and SSCs put into culture (+/-PRP) for 28 days. We analyzed SSC markers (GPR125, CD9, CD49f, CD90, HLA-I, SSEA4) by flow cytometry. We analyzed the ploidy in KS biopsies by DNA FISH.

Main results and the role of chance: We have collected and processed 60 adult testicular biopsies from azoospermic patients as a control, 9 of KS prepuberal patients and 2 of oncologic prepuberal patients.

We have observed that the majority of adult patients, all oncologic prepuberal patients and 20% of Klinefelter Syndrome prepuberal patients express both VASA and MAGEA4 markers by immunofluorescence. In these 20% of KS prepuberal patients that we found previously SSCs then we determined the ploidy (mosaic/pure) by DNA FISH assay. It has been described that GPR125 markers as a putative markers for SSCs in humans. We observed that in our *in vitro* expansion cultures of adult SSCs in the presence of 5% Platelet enriched plasma (PRP) there are more GPR125 + cells (3,3%) in comparison with the control medium (1,5%) condition after 28 days in culture. After these preliminary data in adult SSCs, we will use this novel culture condition for improvement of *in vitro* expansion of prepuberal SSCs.

Limitations, reasons for caution: Due to the limited and difficulty in the obtaining of material used in this study, we used few samples of prepuberal testis.

Wider implications of the findings: We have determined the presence of SSCs in oncologic prepuberal patients (100%) and in SK prepuberal patients (20%). We determined the ploidy (mosaic/pure) of KS SSCs.

The PRP improved the *in vitro* expansion of SSCs in culture during 28 days for future use in auto-transplants to restore patient fertility.

Trial registration number: none

P-811 Fertility preservation in pre-pubertal boys with cancer: cryopreserving immature testicular tissue and developing a three-dimensional scaffolding system to encourage in vitro spermatogenesis

S. Tang[1], J. Celine[1], C. Kevin[1]

[1]University of Oxford, Women's and reproductive health, OXford, United Kingdom

Study question: What are the impacts of three cryopreservation methods on bovine immature testicular tissue (ITT) and can a three-dimensional (3D) scaffolding system encourage *in vitro* spermatogenesis using frozen/thawed tissue?

Summary answer: Speed-controlled slow freezing is favorable for cryopreserving ITT, and spermatogonial stem cell (SSC) colonies can be successfully maintained in a 3D culture system.

What is known already: Annually, around 2000 new cases of childhood cancer are diagnosed in the UK. However, when treated with aggressive chemo- or radio-therapy, prepubertal boys are often rendered infertile. The production of functional sperm from SSCs in the testis is initiated at puberty, thus the cryopreservation of ITT is the only option for pre-pubertal boys at present. In the testis, SSCs are located inside a 3D structure (the seminiferous tubules), and direct cell-to-cell interactions play a critical role in both their proliferation and development.

Study design, size, duration: Three cryopreservation methods (automated slow-freezing, isopropyl alcohol (IPA) uncontrolled slow-freezing, and vitrification) were used to cryopreserve ITT. After thawing, we then investigated apoptosis, gene expression, and the morphology of each sample of ITT. We also isolated SSCs and transferred them into a 3D culture system for further culture.

Participants/materials, setting, methods: Bovine ITTs (n=6) were frozen using three cryopreservation methods. After thawing, ITTs were evaluated by RT-qPCR for germ-cell markers (*PLZF, Oct4, Nanog, Sox2, C-kit* and *Grfa-1*), and apoptosis-related markers (*CREM, KLF4, THY-1, UCHL Stra8* and *HSP70-2*). SSCs were isolated by a two-step enzymatic digestion and Percoll density gradient. Immunocytochemistry, using specific markers (PGP 9.5, PLZF), was then used to detect SSCs, while TUNEL assays were used to determine the extent of apoptosis in testicular cells.

Main results and the role of chance: No significant differences were evident in terms of the expression levels of either germ-cell markers (*PLZF, Oct4,Nanog, Sox2, C-kit,* and *Grfa-1*), or markers of apoptosis (*CREM, KLF4, THY-1, UCHL Stra8,* and *HSP70-2*) when comparing the three methods of cryopreservation. Following the two-step enzymatic digestion of testicular tissue from an immature bovine model, cell viability was 68.87%. SSCs isolated from calf testes exhibited 65% and 59% positivity for PGP9.5 in cells selected from 30% and 40% Percoll gradients, respectively, therefore indicating good rates of SSC recovery. Three days after digestion, TUNEL assays further showed that the apoptosis rates of these SSCs were 21.1% and 14.6% in cells selected by 30% and 40% Percoll density gradients, respectively. Finally, SSCs isolated using a 30% Percoll density gradient showed a significantly higher rate of proliferation ($p<0.05$) than cells isolated from a 40% Percoll gradient. Isolated cells that had been seeded onto Matrigel were successfully able to form SSC colonies, as verified by the positive expression of SSC markers (*PGP 9.5, PLZF*) and the germ-cell marker *Oct4*. The SSC colonies could be maintained in the 3D culture system for up to three weeks.

Limitations, reasons for caution: An increased number of tissues should be tested in order to confirm our findings. Further validation methods are now needed to investigate the function and health of ITT. The time period used for the cryopreservation of ITT could also be a potential source of bias.

Wider implications of the findings: Vitrification represents a potential method with which to cryopreserve ITT since it avoids the need for expensive laboratory equipment.

Trial registration number: not applicable

P-812 The development of a stem cell-based assay for quality control testing in IVF

N. Adjirackor[1], T. Beckitt[2], K. Fowler[1], S. Harvey[1]

[1]Canterbury Christ Church University, School of Human and Life Sciences, Canterbury, United Kingdom ;

[2]Genea Biomedx, Research and Development, Sydney, Australia

Study question: Can embryonic carcinoma cells be used to replace or reduce the use of mouse embryo assays (MEA) in *in vitro* fertilisation (IVF) quality assessment?

Summary answer: Findings from 2D and 3D P19 assays demonstrated the potential of embryonic carcinoma cells to respond to minor changes in culture conditions.

What is known already: The MEA is a toxicological bioassay commonly used to assess the quality of media used in IVF laboratories. Despite its popularity, there are issues with the assay including insensitivity to sub-optimal conditions, ethical concerns about the use of animals, and variation in end-point interpretation between operators. Previous attempts to develop alternative methods have failed because cell and embryo survival have been too high, hence effects that would be toxic to embryos are not detected. There have also been concerns relating to the associated costs and the lack of reproducibility associated with advanced or extended versions of the MEA.

Study design, size, duration: P19 embryonic carcinoma cells (ECACC 95102107) were primed to form 2D (monolayer) or 3D (embryoid bodies) structures in 96-well plates. When induced by DMSO or retinoic acid, P19 cells differentiate into germ layers, and therefore provide a suitable model for embryonic development. Following treatment with test substances, cell viability was determined by Alamar Blue, CellTiter-Glo and RealTime-Glo cell viability assays (Promega), and fluorescence and luminescence outputs were determined using FLUOstar Omega microplate reader.

Participants/materials, setting, methods: Cells were treated with test substances including media containing different concentrations of pyruvate and calcium lactate, two forms of glutamine, and increasing concentrations of base salts. Findings were verified by comparison to treatment with negative (αMEM) and positive (DMSO) controls. In 2D assays, cell viability was determined by fluorescence from resazurin reduction and in 3D assays, by luminescence from Luciferin and NanoLuc reduction. Statistical analyses were performed using ANOVA and Tukey HSD post-hoc tests.

Main results and the role of chance: Both 2D and 3D assays responded to varying effects of energy substrate availability, mono and dipeptide glutamine and increasing osmolality. There were insignificant differences between cellular response to increasing pyruvate and calcium lactate in the 2D assay (pyruvate $F = 0.131$; $df = 6$; $p = 0.717$, calcium lactate $F = 2.803$; $df = 6$; $p = 0.063$), but significant differences in the 3D assay (pyruvate $F = 510.5$;

df = 4; *p* = <0.001, calcium lactate *F* = 1606.71; *df* = 7; *p* = <0.001), indicating increased sensitivity with luminescence-based viability assays in comparison to fluorescence-based assays. Treatment with media containing L-glutamine and alanyl glutamine demonstrated insignificant differences in ammonium build-up between the two forms of glutamine (2D assay *p* = <0.001; 3D assay *p* = <0.001). Findings also showed that cells were sensitive to reduced and increased osmolality, although cell death did not increase with increasing osmolality but rather, complete cell death occurred when media osmolality exceeded 402 mOsmol/kg. Cells were treated with commercially available fertilisation, cleavage and blastocyst media, and comparison to blastocyst development in mice showed equal or increased sensitivity of P19 cells to sequential media, in comparison to MEA findings.

Limitations, reasons for caution: Cellular response to test substances must be directly compared to MEA findings in order to determine the true sensitivity of these cell-based assays. P19 response to commercially available fertilisation, cleavage and blastocyst media are the only set of results that have been directly compared to the MEA.

Wider implications of the findings: This study demonstrates the sensitivity of embryonic carcinoma cells to changing culture conditions, specifically changes in energy substrate levels. The P19 assay could therefore provide a robust, reproducible and cost-effective alternative to MEAs for quality assessment in IVF.

Trial registration number: not applicable

P-813 Extracellular vesicles from endometrial-derived MSCs as coadjuvants for embryo culture mediums: omics and systems biology studies

F. Marinaro[1], M.Á. De Pedro[1], M. Gómez Serrano[2,3,4], I. Jorge[3,4], E. Delgado[5], E. López[1], V. Álvarez[1], J.C. Silla Castro[6], J. Vázquez[3,4], F.M. Sánchez Margallo[3,7], J.G. Casado[1,3]

[1]Jesús Usón Minimally Invasive Surgery Centre, Stem cell therapy unit, Cáceres, Spain ;
[2]Institute of Molecular Biology and Tumor Research- Philipps University, Center for Tumor Biology and Immunology, Marburg, Germany ;
[3]CIBERCV, CIBER de Enfermedades Cardiovasculares, Madrid, Spain ;
[4]Centro Nacional de Investigaciones Cardiovasculares, Laboratory of Cardiovascular Proteomics, Madrid, Spain ;
[5]Clínica Norba Ginecología y Reproducción, Ginecology and Andrology Laboratory, Cáceres, Spain ;
[6]Centro Nacional de Investigaciones Cardiovasculares, Bioinformatics Unit, Madrid, Spain ;
[7]Jesús Usón Minimally Invasive Surgery Centre, Scientific Direction, Cáceres, Spain

Study question: Which is the influence at "system-level" of proteome and miRNAome of extracellular vesicles from endometrial-derived Mesenchymal Stem Cells (EV-endMSCs) on the developing embryo?

Summary answer: Proteins and miRNAs from EV-endMSCs differentially act on embryo cells. Proteins may be involved in immune events and tissue remodelling; miRNAs may regulate cell cycle.

What is known already: It is well known that extracellular vesicles (EVs) have a pivotal role in cell-cell communication. When released by stem cells, EVs act as principal mediators of their potent paracrine effect. Moreover, EVs participate in reproductive processes, spanning from gametogenesis to embryo development. It has been recently demonstrated that EVs from endometrial-derived Mesenchymal Stem Cells (EV-endMSCs) are involved in: I) embryo implantation, II) proliferation of embryonic cells, III) improvement of embryo developmental competence in aged murine models. Nowadays, the molecular mechanisms underlying these findings are still under investigation, especially under a "system biology" point of view.

Study design, size, duration: Endometrial MSCs were isolated from menstrual blood of healthy pre-menopausal women (n=4) and *in vitro* expanded under controlled conditions. EV-endMSCs were collected from cell culture supernatants every 72 hours and concentrated/purified for subsequent analyses.

Participants/materials, setting, methods: Cells and EVs were characterised by flow cytometry for stemness and exosomal markers. Protein extracts were analysed by high-throughput multiplexed quantitative proteomics approach. Total RNAs were isolated and underwent next generation sequencing. Target genes of the mapped miRNAs were identified using miRNet database (https://www.mirnet.ca/). The Reactome analysis tool (https://reactome.org/) was used for enrichment analysis of proteins and miRNAs targets.

Main results and the role of chance: Flow cytometry demonstrated the presence of stemness markers in endometrial MSCs and exosomal surface markers (CD9 and CD63) in EVs.

For proteomic results, a Reactome overrepresentation analysis was performed on a total of 616 proteins (number of peptides ≥ 2, FDR ≥ 0.01). These proteins were also listed under the Gene Ontology category *Extracellular exosome* (GO:0070062). Conversely, miRNAome results were focused on the 6978 target genes of mapped miRNAs (tags per million ≥ 25). In this analysis, only the most relevant pathways (p ≤ 0.05, FDR ≤ 0.01) were considered.

EV-endMSCs proteins appeared to be involved in two major events: *Extracellular matrix organization* (R-HSA-1474244) and *Immune System* (R-HSA-168256). Interestingly, proteins were involved in pathways like adaptive/innate immune system; antigen presentation; neutrophil degranulation; cytokine, interferons and interleukins signalling; platelet activation, signalling, aggregation, and degranulation.

In the case of miRNA target genes, they were found to be involved in the major categories *Cell Cycle* (R-HSA-1640170) and *Gene expression* (R-HSA-74160), actively participating to pathways related to G1 and M phases, cell cycle checkpoints, chromatin organization, pre-mRNA processing, mRNA Splicing, and Translation Initiation. Interestingly, miRNA target genes of EV-endMSCs were also related to the events *Cellular responses to external stimuli* (R-HSA-8953897) and *Apoptosis* (R-HSA-109581).

Limitations, reasons for caution: Although the functional effect of EV-endMSCs has been previously studied in murine embryos, this study was aimed to obtain system biology results. *In vitro* studies including EV-endMSCs-embryos co-culture, followed by immunohistochemistry and qPCR should be performed to confirm the presence of endMSCs proteins and miRNA target genes in embryo cells.

Wider implications of the findings: We hypothesize that EV-endMSCs miRNA cargo may regulate the pre-implantation balance between cell division during embryo cleavage and apoptosis of aneuploid cells in mosaic embryos. Proteins may regulate maternal immune-tolerance and support trophoblast invasion during implantation.

The addition of EV-endMSCs as coadjuvants for embryo culture may increase ART success rate.

Trial registration number: not applicable

P-814 Progress toward manufacturing oocytes

R. Setton[1], A. Trout[1], P. Xie[1], Z. Rosenwaks[1], G. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Can the observation of meiotic-like spindles during somatic cell haploidization provide better artificial oocytes with superior embryonic developmental competence?

Summary answer: Monitoring the appearance of putative meiotic spindles prior to insemination during somatic cell haploidization predicts haploidization and fertilization but not pre-implantation development of reconstructed embryos.

What is known already: Somatic cell haploidization is an in vitro gametogenesis technique that attempts to artificially induce a diploid somatic cell into halving its chromosomal content once incorporated into an enucleated MII oocyte. These newly manufactured oocytes can be fertilized to induce the extrusion of a pseudo-polar body (PB). In studies to date, the most relevant factor for the successful generation of oocytes through haploidization is the ability to choose the right cell at the right cell cycle stage and form a proper meiotic-like spindle prior to the extrusion of a pseudo polar body at the time of insemination.

Study design, size, duration: In our mammalian model, MII oocytes were gathered from superovulated B6D2F1 mice. Meiotic spindles were identified and removed under polarizing light microscope visualization. Nuclear transfer was performed using either a cumulus cell (CC), an embryonic stem cell (ESC), or an endometrial cell (EMC) and fused with enucleated oocytes using Sendai virus. Three hours after nuclear fusion, reconstructed oocytes were

assessed for the detection of a meiotic-like spindle by polarizing light microscopy.
Participants/materials, setting, methods: Oocytes were divided into those that demonstrated a clear meiotic-like spindle and those that did not. Reconstructed oocytes were then fertilized with mouse spermatozoa from the same strain via piezo-actuated intracytoplasmic sperm injection (ICSI). Preimplantation development was compared using a t-test assessing for rates of pseudo-PB extrusion, normal fertilization, and for progression to 2-cell, 4-cell, morula, and blastocyst stages. Each group was also compared to control ICSI conceptuses.
Main results and the role of chance: Of 397 oocytes that were enucleated, 372 (93.7%) survived and underwent transfer with CCs (n=253), ESCs (n=63), or EMCs (n=56). CC nuclei had meiotic-like spindle development at a rate of 59.1% (150/253), ESCs 31.7% (20/63) and EMCs 3.6% (2/56, P<0.0001). Only CC constructs (n=202) underwent ICSI and 111 survived (55%). Haploidization was confirmed by pseudo-PB extrusion in 48.3% (29/60) of reconstructed oocytes with spindles compared to 11/51 (21.6%) that did not (*P*<0.01). Oocytes with spindles yielded superior fertilization (19/60, 31.7%) then those without (5/51, 9.8%, *P*<0.05), but lower than control (88.0%, P<0.0001). When comparing cleavage of fertilized oocytes, 94.7% (18/19) of oocytes with spindles versus 80.0% (4/5) of those without reached 2-cell stage, comparable to control at 83.3%. However, 2-cell stage arrest was prevalent regardless of spindle detection: only 47.4% (9/19) with and 40.0% (2/5) without spindles reached 4-cell stage, a lower rate compared to controls (83.3%, *P*<0.001). Those embryos underwent blastocyst development at a rate of 77.8% (7/9) for those with meiotic-like spindles at insemination and 50.0% (1/2) for those without. All blastocysts (n=8) have been transferred to pseudopregnant mice but did not yield any implantation (0%) in comparison to control (76%).
Limitations, reasons for caution: Somatic cell haploidization remains challenging with unpredictable results. The main limitation is represented by the cell cycle stage of the donor somatic cell nucleus. While visualization of the spindle seemed to predict higher pseudo-PB extrusion and normal fertilization rates, it did not enhance complete pre-implantation development.
Wider implications of the findings: This preliminary data offers satisfactory fertilization and but with compromised embryo growth. The achievement of post-implantation development would set the stage for this technique as an option to generate oocytes for women with exhausted ovarian reserve or primary ovarian insufficiency.
Trial registration number: Not applicable

P-815 Three-dimensional culture of mouse embryonic stem cells maintained on a biological scaffold through direct spherification

S. Lawrence[1], M. Haddad[1], P. Xie[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Does direct spherification provide an alternative method to generate a suitable three-dimensional culture system for stem cells?
Summary answer: Direct spherification provided a sustainable three-dimensional scaffold for growth of embryonic stem cells and allowed their differentiation into early male germ cells.
What is known already: Stem cell research commonly relies on the utilization of a 2D monolayer cell culture partially due to its low cost and high reproducibility. However, 2D cell cultures do not allow the study of 3D intercellular relationships, thus providing an inaccurate replication of *in vivo* environments. 3D culture systems, such as spheroid systems, have been proposed to reproduce the biological microenvironment, particularly when replicating *in vivo* spermatogenesis. Direct spherification, a technique commonly used in molecular gastronomy, provides a cost-effective method to generate a tailored 3D scaffold while allowing fluid exchange across the membrane.
Study design, size, duration: Spheres were made from a base spherification solution of water and sodium alginate. In the first group, mESCs were injected into the spheres; in the second group, a mESC suspension was directly encapsulated (DE). mESC spheres were maintained in a basal medium bath. To coax differentiation, mESC spheres were bathed in epiblast-like cell (EpiLC) medium containing activin A, bFGF, and KSR. EpiLC markers were used to confirm cells at the pre-meiotic germ cell stage.
Participants/materials, setting, methods: MESCs were initially cultured on a 6-well dish and injected into spheres or DE. Each sphere was bathed in mESC or EpiLC medium and incubated at 37°C and 5% CO_2. The diameter of each sphere ranged from 30 to 45 mm, containing approximately $1.2x10^6$ cells per sphere. Viability was tested through cell morphology and reattachment onto inactivated mouse embryonic fibroblasts (MEFs). Differentiation was assessed by using OCT4 and Nanog as markers.
Main results and the role of chance: The morphology of the cellular contents within the spheres showed the survival and growth of mESC colonies with both methods. Spheres injected with mESCs showed a lower concentration of cells compared to the DE approach. Embryoid body formation was first observed in the DE group at day 4, indicating a higher efficiency of this approach. To test the reattachment capability of the mESCs, spheres were mechanically breached to release mESCs, resuspended in fresh basal medium, and plated onto MEF for further propagation. Colony growth and confluency of reattached mESCs were comparable to untreated monolayer mESC culture. Confident with the sustainability and efficiency of DE, we attempted differentiation by bathing the spheres in EpiLC medium. After 3 days of culturing, the spheres were breached and showed a positive OCT4 expression and decreased Nanog expression, indicating successful progression to EpiLCs.
Limitations, reasons for caution: Despite successful sustenance and propagation of mESCs using direct spherification, reproducibility of differentiation and efficiency rates still need to be confirmed. The ability to support differentiation in the post-meiotic stages will provide final proof of reliability of this approach.
Wider implications of the findings: Culturing mESCs inside spheres through direct spherification may provide an alternative method to study intercellular relationships and differentiation in a 3D structure. Incorporation of extracellular matrix proteins during spherification may provide a more physiologically relevant structure for attaining *in vitro* gametogenesis.
Trial registration number: not applicable

P-816 Zygotic cytoplasts of woman of advanced maternal age are competent enough to support normal embryo development when carry young karyoplasts

P. Mazur[1], L. Diachenko[1], V. Veselovskyy[2], Y. Masliy[2], M. Borysov[2], D. Mykytenko[3], V. Zukin[4]

[1]Darwin Life Nadiya, Embryology, Kyiv, Ukraine ;
[2]Clinic of Reproductive Medicine 'NADIYA', Gynaecology, Kyiv, Ukraine ;
[3]Darwin Life Nadiya, Genetics, Kyiv, Ukraine ;
[4]Clinic of Reproductive Medicine 'NADIYA', Administration, Kyiv, Ukraine

Study question: What is the reason of lowering blastulation rates and euploidy rates in women of advanced maternal age (AMA)?
Summary answer: It's plausible that high rates of aneuploidies and low rates of blastulation in women of AMA are associated with the oocyte karyoplast and not cytoplasm.
What is known already: Women of AMA have decreased blastulation and euploidy rates with no regard to their ovarian reserve. It's believed that their oocyte's cytoplasm is responsible for raising of aneuploidies, although, the excact mechanism is unclear. Some of the researchers propose that mitochondria play the crucial role in aging of woman's oocytes.
Study design, size, duration: The study period was from September 2015 to March 2019. Patients were informed and consent to possible risks and the experimental protocol was approved by ethics committee of local association of reproductive medicine. Inclusion criteria were: (1) no less than two failed previous IVF attempts, (2) low blastulation rates or recurrent embryo arrest, (3) low number or absence of euploid embryos, (4) age ≥ 37 years.
Participants/materials, setting, methods: 17 infertile patients (mean age was 41.5±2.5 years) participated in this study. Mean age of oocyte donors was 27.7±3.3 years. Intracytoplasmic sperm injection had been performed in all cases. Pronuclear transplantation (PNT) was assisted by HVJ-E cell fusion kit and reverse reconstitutions (RPNT) were done, if possible. Embryos obtained after reconstitutions were cultured until blastocyst stage in time-lapse incubator, were biopsied for further PGT-A analysis and then were vitrified.
Main results and the role of chance: In PNT group 83 zygotes were obtained and resulted in 25 biopsied blastocysts (30%); 3 of which (one per

patient) were euploid (12%). One try of elective single embryo transfer (eSET) of thawed embryo was done for each of three patients. Positive hCG level (> 100 mIU/mL) and following heartbeating were confirmed only for one patient (42 y.o.).
In RPNT group, where zygotic cytoplasts of women of AMA were used for carrying donor karyoplasts, 58 zygotes were obtained and resulted in 20 biopsied blastocysts (40%); 14 of which were euploid (70%, $p < .05$).
Results in RPNT group are similar to common oocyte donation cycles.
Limitations, reasons for caution: The study was conducted in one center only and the results should be confirmed by other investigators. Also two eSETs (one per patient) of RPNT embryos were done with no implantation, so the real implantation potential of such embryos remains unknown.
Wider implications of the findings: Although the study is limited to obtained zygotes, number of euploid embryos and ongoing pregnancies after applying PNT was low, thereby infertile women of AMA should be advised not to undergone such procedure in order to increase the number of euploid embryos or pregnancy rates.
Trial registration number: None

P-817 An Australian experience of operating IVF clinics during the COVID-19 pandemic

D. Bui[1], S. Dalati[1], S. McArthur[2], N. Hesketh[1], K. Fisk[1], F. Olivier[1]

[1]*Genea, Embryology, Sydney, Australia*
[2]*Genea, Executive, Sydney, Australia*

Study question: How a major Australian IVF clinic managed risk patients and staff to allow a reduction in and subsequent ramping up of assisted reproductive treatments during the COVID-19 pandemic?
Summary answer: Genea implemented a split team working model to enable patients to safely access and staff to safely provide IVF treatment and care during the pandemic.
What is known already: The outbreak of COVID-19 resulted in the declaration of a pandemic by the Australian Government on 27 February 2020 and was followed on March 11, 2020 by the World health Organisation (WHO). These declarations resulted in restrictions being placed on elective surgeries in Australia, including fertility treatment. International response to COVID-19 had at this stage varied from a complete shutdown to a business as usual approach to fertility treatment. Due to a rapid response, Australia managed to significantly slow the spread of the virus, allowing for the staged resumption and increase of elective surgeries, including IVF services.
Study design, size, duration: A review of a work model that enabled social distancing following Australian Government guidance of one person per square metre to reduce the risk of transmission. Half the embryology team was onsite to complete clinical work and perform procedures, with the other half working from home (WFH). Patient facing procedures and discussions were allocated to the WFH team, minimising exposure time of embryologists to patients and to each other.
Participants/materials, setting, methods: Embryologists were rotated one week onsite and one-week WFH, with all patient communication shifted to WFH embryologists. Patient communication was facilitated by utilising Geri time-lapse incubator and Geri Connect. All embryologists were equipped with mobile computer workstations to optimise workflow and communication between embryologists onsite and WFH. Onsite embryologists were required to be temperature tested prior to entering the clinic and laboratory. All clinical embryology was centralised to the group's main clinical facility in Sydney.
Main results and the role of chance: The split team model enabled embryologists to social distance effectively and reduce the risk of virus transmission. Limiting patient facing discussion at the time of clinical procedures reduced the face to face time between embryologists and patients. Implementation of WFH protocols, which included all patient embryology updates, laboratory to laboratory and nurse to embryology communications by telephone and face to face via apps such as Microsoft Teams relieved the onsite embryologists of regular administration work. The onsite embryology team were able to focus solely on delivery of clinical procedures whilst ensuring appropriate levels of patient care were maintained. The adaptability of embryologists was critical to the success of this model due to the rapidly evolving COVID-19 situation. Weekly review meetings were set up to communicate with all embryologists and to discuss and implement best practises as they were identified. WFH protocols were continuously reviewed and refined to improve patient outcomes and service efficiencies. The utilisation of remote access software for time-lapse incubators is central to allowing a split team model to function efficiently.
The split team model also reduced the risk of the whole embryology team being potentially infected with COVID-19 and therefore requiring isolation, effectively shutting down the IVF clinic.
Limitations, reasons for caution: The final analysis of the split team model is limited by the ongoing nature of the COVDI19 pandemic and a final understanding of the benefits and negatives of the split team model are still to be finalised.
Wider implications of the findings: IVF clinics have had to restructure their day to day tasks to continue to provide care to their patients safely during COVID-19. The split team model is one example of how an Australian clinic has been able to safely continue to provide its services to its patients during the pandemic.
Trial registration number: not applicable
Study funding: No
Funding source: Not Applicable

P-818 Vertical transmission of the SARS-CoV-2: an extensive review of the literature

A. Soligo[1], I. Barcelos[2], I. Andrade de Araujo Penna[3], Z. Bernardes Costa[4], W. de Paula Martins[5]

[1]*Universidade de Campinas UNICAMP Brazil/ Assisted reproduction Private clinic, Gynecology and Obstetrics, São Paulo, Brazil*
[2]*Universidade Estadual Oeste Paraná, Gynecology and Obstetrics, Curitiba, Brazil*
[3]*Universidade Federal Fluminense., Gynecology and Obstetrics, Rio de Janeiro, Brazil*
[4]*Universidade Federal de Goiás, Gynecology and Obstetrics, Goiânia, Brazil*
[5]*Faculdade de Medicina de Ribeirão Preto - USP, Gynecology and Obstetrics, Ribeirão Preto, Brazil*

Study question: Is there any evidence on vertical transmission of the SARS-CoV-2?
Summary answer: There is a low probability of vertical transmission.
What is known already: The SARS-CoV-2 coronavirus pandemic has caused an immediate mobilization of the biomedical community with the goal of defining routes of transmission, especially concerning the maternal-fetal condition. In that sense, pregnant women have become a matter of concern due to their susceptibility to respiratory infections, as occurred with MERS and SARS, previously. The route of the viral infection is determined by the affinity of the virus to specific receptors, and the presence of the virus described in the placenta alerts to the possibility of vertical transmission.
Study design, size, duration: A systematic and non-systematic reviews and large observational studies (N > 100) assessing the risk of vertical transmission and perinatal outcomes.

The last electronic search was performed on Apr.30.2020, resulting in total of 1,245 records: We excluded 564 duplicates, and 681 records were screened based on title/abstract resulting in the exclusion of 597 records, as they were not related to vertical transmission of COVID-19. We evaluated the full text of 84 records.
Participants/materials, setting, methods: Not applicable
Main results and the role of chance: Among the systematized reviews eligible to compose our study, a total of 238 pregnancies and 174 deliveries were evaluated, of which the only one suspected vertical transmission case reported above was found, the other 5 cases that we described as suspect included low impact observational studies.

The first case in China, presents a methodology bias, which does not guarantee the occurrence of vertical transmission. In the second one, the diagnosis of possible vertical transmission was made by IgM positivity two hours after delivery, but swab PCR tests was negative. The third, in Iran, the virus in the amniotic fluid was identified by PCR during the cesarean section, but there was no positivity in the nasopharynx samples. This suggests the possibility of perioperative contamination. The fourth reported case, in Peru, showed positivity for the virus by PCR in the sample collected after 16 hours and in the control 48 hours after. Finally, two cases in Italy, where the swab was also not performed shortly after birth and the authors themselves suggest the possibility of postpartum transmission.
Limitations, reasons for caution: Due to the small number of cases included in the analyzed studies, as they are mostly non-randomized retrospectives and without clear selection criteria.

Another weakness of this study is the impossibility of stratifying by gestational age, type of test performed, intertest variation, post-birth maternal confinement and time of neonate examination.
Wider implications of the findings: it is not possible to confirm or refute the existence of any case of vertical transmission of COVID-19 until the date of article selection. Further studies with an appropriate methodological design are needed to establish whether the vertical transmission is an acceptable route and recommend specific protocols.
Trial registration number: Not applicable
Study funding: No
Funding source: Not Applicable

P-819 The dramatic impact of COVID-19 on a busy ART clinic

S. Ahuja[1], E. Fincham[1], M. Taranissi[1]

[1]Assisted Reproduction and Gynaecology Centre ARGC, Reproductive Medicine, London, United Kingdom

Study question: What was the impact of the COVID-19 pandemic on a busy central London ART clinic ?
Summary answer: The COVID-19 pandemic has had a multidimensional impact on all the clinical and functional aspects of the ART clinic.
What is known already: The COVID-19 was declared a pandemic on the 11th march 2020 by WHO. ESHRE, ASRM, BFS and ARCS published guidelines between 14th and 18th March advising clinics to suspend the start of all new fertility treatments, consider cancelling embryo transfers, and suspend elective and non-essential work. HFEA issued general direction 14 on 23rd march, on the same day as lockdown was commenced in UK, to limit treatment to gamete and embryo freezing for those likely to become prematurely infertile ie; oncology patients. They also stipulated that all ongoing treatments should be completed by 15th April.
Study design, size, duration: The impact of the COVID-19 pandemic on the working pattern of the clinic, the clinical management of the patients and the impact on staff, at the time that the fertility societies published guidelines globally, and UK lockdown was introduced, in March 2020, is presented.
Participants/materials, setting, methods: Clinic protocols were modified in line with national guidance on reducing the spread of COVID-19 infection. All patients who were already in the process of fertility treatment or at the preparation stage were advised individually regarding their options informing them about unknown impact on the pregnancy. In addition, due to the suspension of all new fertility treatments, there was a significant impact on all disciplines of staff.
Main results and the role of chance: Clinic pathways were changed to incorporate social distancing, intensified disinfection schedules, the use of PPE and introduction of virtual clinics.

In mid March 2020, there were 46 women aiming for a transfer (31 fresh, 15 frozen). All woman due to have a transfer were counselled regarding their options: cycle cancellation, embryo freezing or proceeding with an embryo transfer. Only 1/46 (2.1%) women due to have an embryo transfer cancelled the cycle due to the lockdown and travel restrictions. There were 26 women expected to start treatment cycles the following week and all their treatments were deferred. In addition, there were about 100 pregnancies (majority as a result of ART) being monitored due to their previous fertility/obstetric history. About 30 % of our staff went on sick leave due to symptoms of COVID-19 in themselves or their family members. A large proportion of the staff were furloughed. A small core team was retained to manage the existing patients.

Inspite of the disastrous effects of the pandemic globally, some positive outcomes included the seamless introduction of virtual clinics, streamlining of SOP's, more efficient working patterns with a smaller core team with personalised care for the remaining patients in this very difficult situation.
Limitations, reasons for caution: The pandemic has been evolving everyday with changes in the advice regarding social isolation, quarantine, testing protocols, the government policies, etc on a regular basis in line with the the available evidence. The above findings highlight the response of our clinic at the time of the epidemic in UK.
Wider implications of the findings: Social distancing measures are likely to continue with virtual clinics, distant teaching/consent, here to stay for the foreseeable future. There is likely to be a surge of patients as soon as treatments are restarted and strict strategies will need to be in place and strictly followed.
Trial registration number: NA
Study funding: No
Funding source: Not Applicable

P-820 Low seroprevalence of SARS Cov2 IgG antibodies in a fertility setting population in Romania

C. Manolea[1], M. Bechir[2], I.A. Rugescu[3]

[1]Carol Davila University of Medicine, IVF Department - Columna Medical Center, Bucharest, Romania
[2]Regina Maria Hospital, Assisted reproduction, Bucharest, Romania
[3]National Transplant Agency, Reproductive Cells, Bucharest, Romania

Study question: Which is the seroprevalence of antibodies directed against SARS Cov2 in a fertility setting population (staff and patients) in Romania?
Summary answer: Only 2% of the people tested demonstrated the presence of IgG antibodies against the novel Coronavirus
What is known already: With a population of slightly over 19 million and 17.700 Covid-19 tests per million people, Romania is amongst the "medium hit" countries, ranking place 37 among 215 countries as regards the number of confirmed infections. As a national particularity, 1 in 7 confirmed cases is a health care worker (HCW).

The lockdown established on the 16th of March was eased starting the 15th of May providing the possibility of restarting the provision of fertility care.

Infertile patients are usually healthy 18 to 45-year-olds, forming a young and active population potentially at risk for developing asymptomatic forms of CoVid-19.
Study design, size, duration: An observational study of a cohort of asymptomatic people – staff and patients of a private fertility unit – consisting of serological testing for SARS Cov2 antibodies (IgM and IgG) undertaken between 11-19th of May 2020.
Participants/materials, setting, methods: We conducted the seroprevalence survey in 100 people (25 HCW and 75 infertile patients) working and respectively attending our private assisted reproduction facility in Bucharest.

The screened persons were aged 25-48 years, living in Bucharest and had a negative epidemiological triage. They have been tested for Ig presence of SARS Cov-2 Ig M and IgG antibodies using an in Vitro chemiluminescence immunoassay (CLIA).
Main results and the role of chance: Out of the 25 HCW tested by CLIA, 1 nurse had a positive result for the presence of SARS-Cov-2 Ig G which means a 4% crude seroprevalence amongst the tested HCW in our clinic.

Regarding the 75 fertility-seeking patients, serological sampling revealed only 1 woman with positive Ig G, so a 1.33% crude seroprevalence of SARSCov2 protective antibodies.

None of the persons undergoing serological testing showed evidence of positive Ig M.
Limitations, reasons for caution: The major limitation lies in the small number of cases, but this is inherent with the short time frame from reopening to the date of abstract submission. It is still a glimpse of a real-life situation in a selected population confined to HCW and patients in a fertility setting
Wider implications of the findings: The seroprevalence of anti-SARS-Cov2 IgGs in this study is consistent with the reported low immunization in the general population. For infertile patients, this could mean that maintaining preventive measures would enhance the safety of restarting treatments.

Knowledge on immunization of fertility HCW could inform decision making in activity organization.
Trial registration number: N/A
Study funding: No
Funding source: Not Applicable

P-821 Patients perspectives on the sudden discontinuation and imminent restart of their fertility treatment due to the COVID-19 pandemic

F. Vanden Meerschaut[1], I. Stuyver[1], K. Tilleman[1], D. Stoop[1]

[1]Ghent University Hospital, Department for Reproductive Medicine, Gent, Belgium

Study question: What are the views and attitudes of fertility patients towards treatment discontinuation and the modalities of treatment resumption during the COVID-19 pandemic?

Summary answer: Patients support the decision to discontinue fertility treatments during lockdown. However, they encourage restart of treatment under strict safety conditions as soon as possible.

What is known already: The COVID-19 pandemic brought unprecedented challenges to healthcare systems worldwide as many countries or cities were put into lockdown with cancellation or postponement of all non-urgent medical care. Consequently, but also precautionary to avoid COVID-19 exposure during (early) pregnancy, leading scientific fertility societies advised for the abrupt discontinuation of most fertility treatments. It has been well established, well before the current pandemic, that fertility treatments lead to significant psychological burden. In that view, the prospect of considerable delays in conception inevitably impacts the patients' wellbeing.

Study design, size, duration: An online anonymous questionnaire, to explore the patient perspectives on the abrupt discontinuation and imminent restart of their fertility treatment, was developed and sent to 1377 patients. A second and third reminder, both after one week, still need to be send. The questionnaire consists of 30 questions and the following answering options: discrete quantitative and nominal categorical variables, scoring questions (Likert scale 1 to 10) and free text fields.

Participants/materials, setting, methods: This study included patients from a university-based fertility center who visited the clinic between January and April 2020. Eligible patients were supposed to start treatment or were undergoing a fertility treatment at the moment of sudden discontinuation of non-urgent hospital activities due to the COVID-19 pandemic.

Main results and the role of chance: Preliminary response rate is 16.1% (n = 222/1377). On a Likert scale of 1 (do not agree) to 10 (fully agree), the mean score for understanding the decision to stop fertility treatment was 7.7 (SD 2.43), whereas when asking if they would have preferred to complete their treatment the score was 6.9 (SD 3.06). The mean score expressing sadness related to the discontinuation of treatment was 7.3 (SD 2.86) (1 = no sadness to 10 = very sad). 24.8% of the respondents believed this delay will have a negative impact on future success rates. When asking if fertility treatment should be prioritized compared with other (non-urgent) medical treatments, the mean score was 6.4 (SD 2.24) (1 = non-priority to 10 = highest priority). Top-5 criteria to prioritize patients for resuming treatment were: female age (83.3%), amount of time being in treatment (55.0%), diagnosis (45.5%), rank of treatment cycle (38.3%) and medical history (32.9%). Respondents were pro safety measures to diminish SARS-CoV-2 transmission during treatment and preferred the following alternative communication tools: telephone call (7.0, SD 2.36), video call (6.3, SD 2.71) and written instructions (6.1, SD 2.61) (1 = do not prefer to 10 = mostly prefer).

Limitations, reasons for caution: Questionnaires cannot fully capture emotional responses or feelings. Although the preliminary response rate is already significant, the results cannot be extrapolated to other centers since the impact of COVID-19 and local healthcare organization might be substantially different.

Wider implications of the findings: Patients suffer from discontinuation of treatment due to the COVID-19 pandemic and are eager to restart treatment in safe conditions. In case a second wave of COVID-19 would require future shutdowns, fertility treatments should be considered as priority medical care, at least for certain groups of patients.

Trial registration number: NCT04396210

Study funding: No

Funding source: Not Applicable

P-822 Status quo and interfering factors of posttraumatic stress disorder symptoms screening in gravidas underwent assisted reproductive technology in the COVID-19 epicenter Wuhan city

M. Wang[1], L. Yang[1], L. Jin[1]

[1]Tongji Hospital- Tongji Medical College- Huazhong University of Science and Technology, Reproductive Medicine Center, Wuhan, China

Study question: Does COVID-19 epidemic cause posttraumatic stress disorder (PTSD) symptoms in gravidas who underwent assisted reproductive technology (ART) in epicenter Wuhan city?

Summary answer: COVID-19 epidemic may increase the risk of PTSD symptoms among gravidas underwent ART in Wuhan city.

What is known already: COVID-19 epidemic has caused hundreds of thousands of deaths worldwide, and Wuhan city was once the epicenter in China. Post-traumatic stress disorder is the most common psychological dysfunction caused by exposure to traumatic events. The health conditions of gravidas underwent ART in Wuhan city during the COVID-19 epidemic deserve attention of reproductive medicine doctors.

Study design, size, duration: A total of 3326 pregnant women who met the inclusion criteria in Reproductive Medicine Center of Tongji Hospital-Tongji Medical College, Huazhong University of Science and Technology.were included. Random sampling was conducted according to the random number table, and 800 questionnaires were given out by our research assistants through the Internet. Participants were asked to accomplish four questionnaires, including PTSD Checklist-Civilian version, Mishel's Uncertainty in Illness Scale-Adult, Simplified Coping Style Questionnaire and Perceived Social Support Scale.

Participants/materials, setting, methods: The PTSD symptom scores of gravidas with different characteristics were compared using non-parametric rank sum test (Mann-Whitney test and Krukal-Wallis test). Multiple linear regression was used to analyze the influence of different characteristics of gravidas on PTSD symptom score. A structural equation model was established to analyze the path analysis of PTSD symptoms with disease uncertainty, social support and coping style.

Main results and the role of chance: A total of 488 gravidas who visited Reproductive Medicine Center of Tongji Hospital-Tongji Medical College, Huazhong University of Science and Technology during April 2019 to January 2020 were included in this study. 12.3% of them were considered to be high-risk of PTSD during COVID-19 epidemic. The result of multiple linear regression showed that a history of chronic disease and the frequency of checking the epidemic situation were positively related with PTSD symptom scores, but pregnancy complications, sleep quality, whether relatives were isolated, fear of infection, confidence in current prevention methods, and bleeding or abdominal pain during the closure period were negatively related with PTSD symptom scores. In addition, uncertainty in illness had a positive effect on PTSD directly or through coping styles, while perceived social support affect PTSD negatively completely through coping styles.

Limitations, reasons for caution: Firstly, coping style was the only intermediary variable analyzed in this study. Secondly, due to the epidemic situation, we failed to carry out psychiatric examination for patients with positive PTSD symptoms. Finally, the live birth rate and neonatal complications in PTSD symptoms gravidas are needed follow-up investigation.

Wider implications of the findings: Medical workers should pay more attention to mental health of gravidas underwent ART during the epidemic of COVID-19. PTSD symptoms may be relieved by reducing the uncertainty in illness, giving sufficient social support and strengthening positive coping styles.

Trial registration number: not applicable

Study funding: Yes

Funding source: Funding by national/international organization(s)

P-823 COVID-19 among assisted reproduction patients in Spain. What should we do to stop the spread of infection

G. Barrenetxea Ziarrusta[1], I. Romero[1], R. Celis[2], A. Abio[3], M. Bilbao[4], J.I. Arambarri[5], J. Barrenetxea[6]

[1]REPRODUCCION BILBAO, Gynecolgy & Assisted Reproduction, BILBAO, Spain
[2]Reproduccion Bilbao, Gynecology & Assister Reproduction, Bilbao, Spain
[3]Reproduccion Bilbao, Gynecology & Assisted Reproduction, Bilbao, Spain
[4]Reproduccion Bilbao, Gynecology & asisted Reproduction, Bilbao, Spain
[5]Reproduccion Bilbao, Gynecology & Obstetrics, Bilbao, Spain
[6]Osakidetza, Gynecology and Obstetrics, Bilbao, Spain

Study question: Once assisted reproduction (AR) treatments are reinitiated, is it enough to screen patients and staff by clinical means or should we assess them more thoroughly?

Summary answer: From a public health perspective, detection and quick isolation of asymptomatic patients is of paramount importance to discontinue the spread of the disease.

What is known already: Up to 18th of May 2020, almost 5,000,000 cases with COVID-19 were diagnosed and more than 300,000 death cases were recorded.

It has been stated by ESHRE and other organizations that any risk of viral contamination from SARS-CoV-2 to gamete and embryos in the IVF laboratory is likely to be minimal and pregnant women should not be considered at higher risk for developing severe infection,

Detection of asymptomatic patients, who can spread the disease, is of paramount importance.

Presently, RT-PCR for RNA detection is the only acknowledged method to rapidly and accurately detect COVID-19 infection in humans.

Study design, size, duration: The study was carried out from May 4th to 18th (after restart of clinical activity at AR centers).

Observational study involving 194 infertility patients and 19 staff members of an AR center. Data are reported as percentages. A chi-squared test was used for comparisons of qualitative variables (significance set at p<0,05).

The study was approved by Institutional Review Board . All the procedures were in accordance with the Helsinki Declaration of 1964 and later amendments.

Participants/materials, setting, methods: First, a clinical and epidemiological survey was completed by both, patients and staff members.

Second, those patients scheduled for an ovarian puncture for egg retrieval and/or embryo transfer were assessed by a double diagnostic test: a RT-PCR to detect SARS-CoV-2 RNA from nasopharyngeal aspirate and serologic test to determine the presence of, either, IgG or IgM against the virus. The double test was also performed on the partners of the patients.

Main results and the role of chance: Sixty-eight and 19 RT-PCR and serologic immunoglobulins determinations were performed among patients and staff members respectively.

Among patients, 30,4% were confined at home during lockdown, 27,8% were teleworking, 25,8% were working at the office, whereas 16 % were on sick leave (from diseases different of COVID-19).

Five patients reported previous symptoms like fever, cough, diarrhea, muscle aches, in the past two weeks. However, only 2 out of these patients had been previously offered to perform any diagnostic test. No symptomatic patient had positive RT-PCR or serologic results at our setting. Among 189 asymptomatic patients, 2 (1,1%) had positive serologic determination of IgG and/or IgM. No patient had a positive RT-PCR determination.

Even though 12 (6,2%) of the patients reported that a relative had been diagnosed of COVID-19 only one of them (8,3%) had had any diagnostic test performed prior to attending at our center.

There was a positive RT-PCR case among staff members (5,6%). It was an asymptomatic case with positive determination of IgG and IgM. The test was repeated after an asymptomatic period of 3 weeks and the virus was not cleared. The patient remains asymptomatic. A new test is to be performed in 3 weeks-time.

Limitations, reasons for caution: The results in our series show a low percentage of positive RT-PCR and a low presence of antibodies against SARS-CoV-2.

Although this figure may be due to the lockdown itself, it may show that the assessed patients do not represent properly general population.

Wider implications of the findings: Even though the low percentage of positive cases, we should take into account that asymptomatic patients may be infected by the SARS-CoV-2 and, thus, may spread the infection.

The only positive cases in our series were asymptomatic, highlighting the importance of performing the PCR and serologic tests.

Trial registration number: non-applicable

Study funding: No

Funding source: Not Applicable

P-824 Psychological themes with restarting fertility treatment in the era of Covid-19: A tertiary centre

A. Pericleous-Smith[1], M. Mascarenhas[1], H. Bhandari[1]

[1]Leeds Teaching Hospitals, Leeds Fertility, Leeds, United Kingdom

Study question: What are the psychological experiences of men and women on learning that fertility treatments are being restarted after they were suspended in view of Covid-19?

Summary answer: Whilst most expressed positive emotions on learning of the restart of fertility treatment, a substantial proportion expressed ambivalence or a wish to defer fertility treatment

What is known already: Infertility and the unpredictability of the outcome of fertility treatment are known psychological stressors. The Covid-19 pandemic has led to an unprecedented suspension of fertility treatment across Europe. The suspension of fertility treatment and the uncertainty as to when this suspension might be lifted has created an additional psychological burden on men and women seeking fertility assistance. There has been much recent research and attention in the lay-press on this psychological impact – especially on those whose biological clock is 'ticking'. Fertility treatment suspension is gradually being lifted in some European countries, depending on local control of the pandemic.

Study design, size, duration: Remote counselling sessions of 60 minutes duration with both individuals and couples were carried out between 1st May 2020, on the day the UK government and the HFEA announced that fertility clinics can resume fertility treatment. Data available until the 20th May 2020 from 48 counselling sessions (including 31 individual and 17 couple counselling sessions) is summarised here with further data to be available at the time of presentation.

Participants/materials, setting, methods: Thirty-five participants were married, six unmarried, but in stable relationships and seven single women. Counselling offers a safe place for patients to explore any uncertainties and manage expectations. Sessions were undertaken remotely via telephone (n = 34; 71%) or video (n = 14; 29%) consultation. Each session included open questions of the government's announcement and participants shared their experiences, perceptions and concerns of clinics re-opening including any communication they had received from their clinic.

Main results and the role of chance: The 48 participants were aged between 18 and 48 years (average age 34.11 years). Some (31%) participants reported the announcement as good news, 69% expressed uncertainties, ambivalences and concerns and one participant reported suicidal ideation.

Some patients believed treatment would resume on 11th May 2020 (date from when clinics could apply to resume treatment). Clarification was provided, which resulted in patients' perceptions of time frames ranging from "with next cycle", "in 4-6 weeks", to "seven months".

The majority (n = 27; 56%) reported having attempted to contact the clinic. Patients felt coping strategies, stress and anxiety could be better managed if they had (i) contact from the clinic and (ii) clearer time frame to resume their individual treatment.

Those with pre-existing relationships with clinic staff expressed concerns over relational dynamics with the use of personal protective equipment and partners unable to be present for appointments.

Several raised anxieties in relation to safety of pregnancy and impact of second pandemic wave on their treatment. Some reported changes in financial circumstances affecting their ability to proceed with treatment.

A few reported waiting "to see" how clinics and the pandemic "pan out" with three couples planning to delay by at least 1 year.

Limitations, reasons for caution: The sample size is limited, and the profile of patients who choose to have counselling might not be the same as the overall population of patients seeking fertility treatment. Therefore, our findings should be taken as descriptive and may not be suitable for extrapolation to the outpatient clinic setting.

Wider implications of the findings: Most patients had positive thoughts and emotions on hearing the news that fertility treatments could recommence. Clinics should take note that patients felt that contact initiated by the clinic rather than patients regarding restarting treatment and providing better time scales might help to manage their stress and anxiety.

Trial registration number: not applicable

Study funding: No

Funding source: Not Applicable

P-825 Impact of COVID19 pandemic on fertility treatment : a Tunisian Experience

H. ELLOUMI[1], M. Ben khelifa[1], S. Mnallah[1], M. Khrouf[2], K. Terras[2], M.H. Ben aribia[1], F. Zhioua[2], M. Benkhalifa[1], K. Mahmoud[2]

[1]fertility center FERTILLIA- Tunis- Tunisia., Laboratory of reproductive biology, tunis, Tunisia

[2]fertility center FERTILLIA- Tunis- Tunisia., Gynecology departement of the fertility center FERTILLIA, tunis, Tunisia

Study question: Here we aim to investigate the impact of the current covid-19 pandemic on patients who underwent reproductive treatment compared to COVID19 pre-pandemic period

Summary answer: The current COVID19 pandemic has a slight negative effect on outcome fertility treatments

What is known already: During the COVID19 pandemic, strict measures have been adopted by tunisian governement, and as a result we have observed a slow progression of the disease. So, we have observed radical changes in health care practices. Reproductive Medicine Societies have advised people not to undergo infertility treatment . The lockdown of ART centers was detrimental for society as a whole, and infertility patients in particular. In our ART clinic, the laboratory provided limited service for the emergency procedures, and for patients in active treatment cycles.

Study design, size, duration: Retrospective descriptive study evaluating activity during COVID19 pandemic (Group A) and pre-pandemic period (Group B) in an ART clinic. In this period (March and April 2020), we had remaining activity concerning the following acts : intrauterine insemination (IUI), In vitro fertilisation (IVF) and frozen-thawed embryo transfer (FET). We screened all patients for exposure and risk factors for COVID19 before being authorized to join ART unit. Treatment of Patients who failed screening, was cancelled.

Participants/materials, setting, methods: About IVF cycles, the freeze-all strategy was proposed to all couples, but some couples insisted on fresh embryo transfer. All transfers were made on day 3 post-pickup. In all cases, the couple signed a consent form. An online survey was sent to our current patients to estimate psychological COVID19 pandemic effect and outcomes of infertility treatment. All statistical analyses were performed using SPSS software. A significant difference was accepted when the p value was <0.05.

Main results and the role of chance: A total number of listed acts were performed during pandemic and prepandemic period respectively: IUI cycles: 27 vs 49 ; IVF cycles:202 vs 450 and FET cycles: 60 vs 120. Four couples could not have access to our ART center because they failed screening for exposure and risk factors for COVID19. We called all patients who had treatment during COVID19 pandemic in our center, two and four weeks later, and no patients became sick from COVID19. The online survey indicate that 80% of respondents found that pandemic situations have an important emotional impact with 50% rating it to be equivalent to loss of chance to conceive. Concerning IUI cycles, no signifiant difference was observed of CPR between the two groups (A:13% ; B:12.5% ; p<0.05). Using an intention to analyse IVF cycles data, 56% of couples only accepted a freeze all strategy. We identified significant diffrence concerning Clinical pregnancy rate, and miscarriage rate in groups A and B were (28.5% vs 32.9 % ; 29% vs 24%) respectively (p<0.05). Parameters of the ovarian stimulation were comparable between the two groups. Concerning FET cycles no signifiant difference was observed in terms of CPR and MR between the two groups .

Limitations, reasons for caution: This retrospective data set included a variety of treatment and protocols used in two periods with different characteristics. In addition, the rates observed concerned all the patients treated, the comparison of rates between treatments, should be subject to further investigation

Wider implications of the findings: Given the severity of the COVID19 pandemic, the physical and emotional impact of this unprecedented threat cannot be underestimated in our fertility patients.

Trial registration number: not applicable

Study funding: No

Funding source: Not Applicable

P-826 Fertility patients under COVID-19: Attitudes, Perceptions, and Psychological Reactions

R. Ben-Kimhy[1], M. Youngster[2], T.R. Medina-Artom[3], S. Avraham[2], I. Gat[2], L. Marom Haham[4], A. Hourvitz[2], A. Kedem[2]

[1]Meir Sapir Medical Center, IVF Unit- Department of Obstetrics & Gynecology, Kfar-Saba, Israel

[2]Shamir Medical Center, Reproductive endocrinology- Obstetrics And Gynecology, Zrifin, Israel

[3]Tel Aviv University, The Bob Shapell School of Social Work, Tel Aviv, Israel

[4]CReATe Fertility Center, Fertility Unit, Toronto- Ontario, Canada

Study question: What were the perceptions of infertility patients and the factors contributing to their psychological distress, following suspension of fertility treatments during the COVID-19 pandemic?

Summary answer: Higher self-mastery and greater perceived social support were associated with lower distress. Feeling helpless was associated with higher distress. Background characteristics had no significant contribution.

What is known already: Infertility diagnosis and treatment frequently result in significant psychological distress. Personal resources play an important protective role in times of crisis, helping to reduce levels of distress. This is the first study, to the best of our knowledge, to describe the psychological impact of global suspension of fertility treatments and provide a window to patient's state of mind in this particular complex time.

Study design, size, duration: Cross-sectional questionnaire study including patients in a tertiary hospital, whose fertility treatment was suspended following the COVID-19 pandemic. The survey has been delivered to 297 patients within 12 days at the beginning of April 2020

Participants/materials, setting, methods: The self-administered questionnaire included items addressing: 1. Patient's demographic characteristics, 2. Anxiety related to COVID-19 infection risk and level of social support (COVID-19 anxiety score and social support score were calculated), 3. Patient's perception of the new guidelines and description of subsequently related emotions, and 4. Two validated scales assessing levels of emotional distress (MHI-5) and Self-mastery. Multivariate analysis was conducted to assess factors alleviating or exacerbating emotional distress during the COVID-19 pandemic.

Main results and the role of chance: One hundred sixty-eight patients completed the survey for a response rate of 57%. Study variables in the regression model explained 38.6% of the variance in psychological distress experienced by patients during treatment suspension. None of the background characteristics (e.g. age, marital status, economic level, duration of treatments, and parity) had a significant contribution. Higher self-mastery and greater perceived social support were associated with lower distress (p<0.01). Feeling helpless following the suspension of treatments was associated with higher distress (P<0.01). Seventy two percent of patients wished to resume treatment at the time of survey despite the ministry of health's decision.

Limitations, reasons for caution: This pandemic, its length and implications are unknown, therefore, the ability to draw conclusions about the psychological consequences of the crisis is limited at this point of time. The study was conducted in one IVF unit which may influence its generalizability, however, the high response rate of 56.6%, enabled heterogeneity.

Wider implications of the findings: Study findings suggest that attention should be paid to strengthening and empowering patients' personal resources together with directly confronting and containing feelings of helplessness. In line with the ESHRE guidelines, especially at time of high levels of distress, it is imperative for caregivers to offer emotional support.

Trial registration number: not applicable

Study funding: No

Funding source: Not Applicable