Review: Semen extenders used in the artificial insemination of swine

J. Gadea

Departamento de Fisiología. Facultad de Veterinaria. Universidad de Murcia.
Campus de Espinardo. 30071 Murcia. Spain

Abstract

This report reviews the latest knowledge on boar semen diluents used in artificial insemination procedures. The requirements of an effective fresh semen diluent are discussed and currently available extenders are compared. Finally, we suggest directions to be taken in future research on this topic. A semen extender should be carefully selected according to its proposed use. For a planned semen storage time lower than 72 h, it is preferable to use a short-term extender. This type of diluent is less expensive and has been associated with a similar reproduction outcome to that of long-term extenders. When semen doses need to be stored for more than 4 days (because of long distances, disease control procedures, etc.), we recommend the use of a long-term extender on a higher sperm concentration to compensate for reduced sperm viability due to ageing. The semen extender must be selected on the grounds of optimal performance (in terms of fertility and litter size), which, in turn, depends on the particular conditions of each pig farm. The choice of extender is crucial given its profound effect on the economic viability of the artificial insemination programme.

Key words: artificial insemination, swine, diluent, spermatozoon, fertility.

Resumen

Revisión: Los diluyentes de inseminación artificial porcina

Nuestro objetivo fue revisar a la luz de los últimos trabajos publicados los requisitos de un diluyente de inseminación, la importancia de la elección del mismo en las condiciones de producción actual y apuntar hacia dónde van dirigidas las investigaciones para su aplicación en un futuro próximo. Asumiendo que no hay un diluyente perfecto, su elección debe ir asociada al tipo de uso que se vaya a hacer de él. Cuando el tiempo de conservación sea inferior a tres días, la elección más racional sería la utilización de un diluyente de corta duración con unos costes menores y con unos resultados equivalentes a los de diluyentes de larga duración. Cuando lo que se pretende es conservar dosis seminales más allá de 4 días (debido a las largas distancias, evaluaciones sanitarias del semen, etc) se deben utilizar diluyentes de larga duración y aumentar la concentración de la dosis para compensar las pérdidas por envejecimiento de los espermatozoides. En cualquier caso la elección del diluyente debe realizarse con el objetivo de optimizar los resultados de fertilidad y prolificidad en las condiciones particulares de cada explotación porcina, ya que su repercusión en el rendimiento económico de la explotación es crucial.

Palabras clave: inseminación artificial, porcino, diluyente, espermatozoide, fertilidad.

Introduction

The artificial insemination (AI) of the pig was first performed by Ivanow in Russia at the start of the XXth century (Ivanow, 1907; 1922). In the 1930s, the procedure was developed at Russian state farms (Rodin and Lipatov, 1935; Milovanow, 1938) and over subsequent years, the practice of AI spread to other countries (USA, Mckenzie, 1931; Japan, Ito *et al.*, 1948). Following the studies performed by Chris Polge (1956), AI was reintroduced for swine production in the UK. The great advantage of AI is that the genetic potential of the best boars can be transferred to a large number of sows, leading to genetic improvements. However, the true development and wide commercial application of AI in pig production did not take place until the 1980s (reviewed in Reed, 1985; Crabo, 1990; Johnson *et al.*, 2000) when insemination protocols were standardised. This almost entire century of deve-

lopment has obviously also seen considerable advances in methods of collecting the semen from boars and preparing these semen doses, as well as continued progress in designing AI protocols for use in commercial settings.

Today, AI in pig production is widely applied throughout the developed world, although its extent of use in the different countries is highly variable. In Europe, this reproduction technique is generally extensively used, accounting for 80% of the reproductive gilts/sows in many countries (Holland, France, Germany, Spain, Norway, Finland, etc.). In contrast, the rate of AI use in the US is still low (around 50%), though these last few years have witnessed a notable increase. According to most recent estimates, some 19 million inseminations are performed world-wide per year, of which almost all (99%) are conducted using boar semen preserved at a temperature of 15-20ºC (Johnson *et al.*, 2000). Over 85% of these artificial inseminations are performed on the day or following day of sperm collection. The development of the AI technique in pig production has been mostly prompted by the dissemination of improved boar genes and the fact that results are equivalent or even better than those related to natural service.

This report analyses the economic and productive implications of the diluents used in porcine AI. Despite the established importance of the type of semen diluent used, this topic has not been extensively reviewed in the literature (Weitze, 1990; Reed, 1990; Althouse, 1997; Johnson, 2000; Levis, 2000). Our aim was thus to review —in the light of the most recently published findings— the significance of the choice of semen diluent in current conditions of pig production.

Diluents

By the term diluent, or extender, we mean the aqueous solution used to increase the volume of the ejaculate until that of the required dose. This needs to be done while preserving the functional characteristics of the sperm cells such that the appropriate sow fertility rate is maintained.

The spermatozoa are found in the seminal plasma, which supplies them with the necessary nutrients for the high metabolic demands of sperm transport through the female genital tract. In the ejaculate, this high metabolic activity can only be maintained over a limited period, as established in early studies on the preserva-

tion of boar semen (Lewis, 1911). Thus, to preserve spermatozoa for prolonged periods, their metabolic activity needs to be reduced by diluting it in an appropriate medium and lowering the temperature.

Given their particular features, boar spermatozoa are extremely sensitive to cold shock (Pursel *et al.*, 1973a) which alters sperm viability. Specifically, this sensitivity seems to be related to the lipid content of the sperm cell membranes. Thus, when the temperature falls, lateral movements of membrane phospholipids are reduced and this causes separation of the lipid phases, which is associated with irreversible alterations to membrane proteins. The end result is that the function of the sperm membrane changes, compromising cell viability (reviewed in White, 1993). This reaction to cold shock means that in practice, semen samples need to be kept at 15-20ºC, since a further reduced storage temperature limits their viability (Paulenz *et al.*, 2000).

The need to preserve boar semen samples at these moderately reduced temperatures restricts their storage capacity since cell metabolism cannot be slowed down and because microbiological conditions may not be as effectively controlled as at lower temperatures (5ºC).

In addition, dilution lowers the concentration of certain compounds in the seminal plasma, such as K^+ (Harrison *et al.*, 1978) or plasma proteins, altering sperm viability. These losses need to be compensated by adding ingredients to the diluent formulation such as bovine serum albumin (BSA), which has been shown to enhance motility (Waberski *et al.*, 1989) and improve fertility rates derived from the use of preserved semen (Waberski *et al.*, 1994a).

Types of diluent

At a practical level and for current production purposes, diluents can be divided into two major groups: those designed for short-term preservation (less than 1-3 days), and diluents for long-term semen preservation (over 4 days) (Table 1). The former are mainly used in short distance semen dose distribution networks (such as European systems in which semen doses are frequently produced at the farm itself), while long-term diluents are generally used in programmes such as those of the US or Norway, where the site of semen production is a long distance away from the site of insemination.

Table 1. Diluents classed as short- and long-term

Short-term (1-3 days)	Long-term (over 4 days)
Beltsville Liquid (BL-1)	Acromax®
Beltsville Thawing Solution (BTS)	Androhep®
Illinois Variable Temperature (IVT)	Modena
Kiev	MR-A®
Vital®	MULBERRY III®
	Reading
	X-Cell®
	Zorlesco
	ZORPVA

The advantages of long-term diluents include the possibility of: long distance transport, conducting diagnostic tests on semen before use, such as the polymerase chain reaction (PCR) to detect the presence of several viruses or a full analysis of semen quality, improving the organisation of tasks at semen collection centres and —to a large extent— this type of diluent helps distribute the semen samples to the reproduction farms.

The first Russian diluents were based on glucose solutions containing sodium or potassium tartrate or sodium sulphate and peptones, always ensuring electrolyte levels were low (reviewed in Foote, 2002a). Since then, in the 1950s, diluents for use in cow produc-

tion based on egg yolk plus phosphate or citrate and milk were developed, and certain modifications for preserving boar semen were adopted (reviewed in Foote, 2002a). Of note among these was the adapted Illinois Variable Temperature diluent, which was used to preserve cattle semen at room temperature (du Mesnil du Buisson and Dauzier, 1959). The IVT medium is based on a solution of glucose, citrate, bicarbonate and egg yolk, but needs to be gassed with CO_2 to lower metabolic activity (Table 2).

The great innovation of the 1960s was the addition of a chelating agent (EDTA) to semen extenders, to block the action of calcium as a mediator of sperm capacitation and the acrosome reaction. It was at this time that the Kiev diluent appeared on the scene (Plisko, 1965) and subsequently became modified under several names (EDTA, Merck I, Plisko, Guelph). The Kiev diluent was responsible for the expansion of the AI of swine and is still successfully used today.

The exhaustive work performed at the Beltsville Center (USA) on possible methods of preserving boar sperm was the highlight of research on the topic in the 1970s. The team headed by Pursel and Johnson undertook numerous tests to develop diluents for cold storage (BL-1) (Pursel *et al.*, 1973b) and freezing (BF-5) (Pursel and Johnson, 1975). Undoubtedly, their most far-reaching discovery was that of the BTS (Bets-

Table 2. Composition of the most commonly used boar semen extenders

Composition (g L⁻¹)	IVT	Kiev	BTS	Zorlesco	MRA	ZORPVA	Reading	Modena	Androhep
Glucose	3	60	37	11.5	+	11.5	11.5	25ᵃ	26
Sodium citrate	24.3	3.7	6.0	11.7	+	11.65	11.65	6.90	8.0
EDTA		3.7	1.25	2.3	+	2.35	2.35	2.25	2.4
Sodium bicarbonate	2.4	1.2	1.25	1.25	+	1.75	1.75	1.00	1.2
Potassium chloride	0.4		0.75		–		0.75		
Acetylcysteine	0.05								
Hepes									9.0
BSA				5.0	+			3.00	2.5
Tris				6.5	–	5.5	5.5	5.65	
Citrate				4.1	–	4.1	4.1	2.00	
Cysteine				0.1	+	0.7	0.7	0.05	
Trehalose								1	
PVA						1	1		
Potassium acetate					+				
MOPS					+				
mOsm	290	380	330	240	290	275	300	282	309
pH		7.2	7.2		6.9			6.9	6.8

ᵃ: glucose monohydrate. BTS (Pursel and Johnson, 1975); Zorlesco (Gottardi *et al.,* 1980); IVT (du Mesnil du Buisson and Dauzier, 1959); Kiev (Plisko, 1965); Modena (Moretti, 1981); Androhep (Weitze 1990); MR-A (Martín Rillo, 1984); ZORPVA (Cheng, 1985); Reading (Revell and Gossop, 1989).

ville Thawing Solution) medium (Pursel and Johnson, 1975), initially designed as a thawing medium and subsequently adapted for refrigerated semen (Johnson *et al.*, 1988). BTS, probably the most widely used semen extender throughout the world, is characterised by containing a small amount of potassium. This feature preserves the sodium potassium pump and thus avoids intracellular potassium depletion which is related to reduced sperm motility (Alvarez and Storey, 1982).

The first of the so-called long-term diluents was the Zorlesco medium (Gottardi *et al.*, 1980). This fairly complex medium is composed of Tris as a pH regulator, bovine serum albumin (BSA) and the amino acid cysteine. As other compounds with a sulphydryl group, cysteine is a membrane stabiliser and capacitation inhibitor (Johnson *et al.*, 2000). The use of this diluent in field conditions yielded poor results (Table 2), partly because of imbalances in its composition that lead to a low final osmotic pressure (240 mOsm). This was followed by the creation of the Modena diluent by Moretti (1981), who increased the proportion of glucose and removed the BSA of the Zorlesco medium only to find that fertility results were still unsatisfactory (Johnson *et al.*, 1988; Laforest and Allard, 1996).

In a concurrent manner, Martín-Rillo and Alias developed the MR-A medium in Spain (Martín Rillo, 1984), and although its composition has not been disclosed to the public for commercial reasons, its performance as a long-term diluent up until now has been good.

In this same period, two long-term diluents were designed in the UK: ZORPVA (Cheng, 1985) and Reading (Revell and Gossop, 1989). Both are complex media based on a slightly modified Zorlesco medium in that they contain polyvinyl alcohol (PVA) as a macromolecule, improving the proportion of intact acrosomes. These diluents are more expensive (149-163%) than their short-term counterparts and their results do not surpass those obtained with other diluents (Reed and Curnock, 1990). It is due to these high costs, that their use has not become widely extended.

Weitze (1990) developed the Androhep diluent, comprised of Hepes as pH regulator and BSA to compensate for the dilution effect on seminal plasma proteins and for its slightly hypertonic nature (309 mOsm). This diluent has been well accepted in the swine production sector as a long-term semen extender.

Over the last few years, several new long-term diluents have emerged, as Acromax, X-Cell, Androhep Plus, Vital, SpermAid, Mulberry III, Safe Cell Plus,

etc. Unfortunately, the quantitative composition of these media is unknown due to commercial interests, and though we do not doubt the quality of these diluents there is a lack of fertility data derived from comparative studies conducted at independent centres. Hence, we will need to wait for this information to become available.

Diluents used for freezing boar semen are based on egg yolk and glycerol as cryoprotecting agents, a high concentration of sugars and a detergent (Orvus et paste). The most commonly used diluents are the lactose-egg yolk medium (Westendorf *et al.*, 1975) and the extender denominated BF-5 described by Pursel and Johnson (1975), whose composition includes glucose, egg yolk, and Tris as pH regulator. The BF-5 medium is used in freezing processes performed on semen pellets on dry ice.

Diluent actions

To perform its function, the extender should supply the nutrients needed for the metabolic maintenance of the sperm cell (glucose), afford protection against cold shock (BSA), control the pH (bicarbonate, Tris, Hepes) and osmotic pressure (NaCl, KCl) of the medium, and inhibit microbial growth (antibiotics).

Nutrients

The spermatozoon can produce the energy needed to maintain its cell metabolism and cause the flagellum to move, mainly through glycolytic pathways. These processes occur in the mitochondria located in the middle portion of the spermatozoon. The source of energy most commonly used in semen diluents is glucose, although other sugars have been tested (galactose, fructose, ribose or trehalose) but have generally yielded worse results.

Regulating pH

The pH of freshly ejaculated boar semen is around 7.4±0.2, similar to other body fluids. When this pH is reduced, both the sperm's metabolism and motility are reduced. Its glycolytic metabolism (glucose is the main carbohydrate) leads to a reduced intracellular pH and consequently cell metabolism is suppressed. Lactic acid

is the main metabolite of this process and has been used as an indicator of semen quality (Rigau *et al.*, 1996).

The addition of buffering agents therefore helps control the pH of the medium. The simplest buffers used are bicarbonate and sodium citrate, which show a limited buffering capacity. Other more complex buffers (TES, Hepes, MOPS, Tris) can control the pH over a wider range and are not temperature-dependent (MOPS and Hepes).

The pH of the diluents normally used ranges from 6.8 to 7.2 (Table 2), but it should be taken into account that in these media, the pH does not become stable until 60-90 min from the start of dilution in water and that the different extenders show a different pattern of pH change over time (Newth and Levis, 1999). Thus, appropriate measures need to be taken when preparing the diluent to avoid detrimental effects on preservation.

Osmotic pressure

The boar spermatozoon has an osmotic pressure of 290-300 mOsm, and can tolerate a fairly wide range of osmotic pressures (240-380 mOsm). Several authors have evaluated tolerance to different osmotic pressures, and concluded that neither motility nor viability are affected by osmotic pressures in the range 250 to 290 mOsm (Fraser *et al.*, 2001). However, at pressures below 200 mOsm, motility is significantly reduced (Gilmore *et al.*, 1996, Fraser *et al.*, 2001).

Isotonic or slightly hypertonic diluents (300 mOsm) have provided best results in conditions of commercial use. Salts of inorganic ions such as sodium and potassium chloride are mainly used to regulate osmotic pressure.

Diluent costs

In general, costs related to reproduction represent a low proportion of the overall cost of pig production and have been estimated at 1.9% (Rouco and Muñoz, 1998). If we consider that most of this cost is associated with the human resources needed for diagnosing oestrus, semen collection and administering semen doses (Flowers and Esbenshade, 1993; See, 1996), the diluent represents an insignificant portion of complete production costs. Nonetheless, at artificial insemination centres that market semen doses, the choice of diluent does of course have its economic repercussions.

Whichever the case, the cost of the extender is negligible compared to the possible consequences on farrowing rates and litter sizes.

The concept of diluent costs should not only include those needed to purchase its components but also those derived from its preparation. Thus, over the past few years, owing to high personnel costs, many laboratories opt for the use of powdered preparations (or concentrated liquid stock solutions) that only require diluting in distilled water. This method contrasts with in-house preparation, which involves separately weighing each component before final dilution.

The emergence of several firms that design and produce their own diluents has greatly extended the range of products offered and has given rise to a successful market and commercial activity (Pig International, 1998) in which there is intense competition among distributors. It may be said that, in general, long-term diluents are more expensive to produce than short-term ones, due to higher cost constituents such as Tris, BSA, etc., and the longer time needed to weigh the higher number of components (Reed, 1990).

Use of antibiotics

In most cases, the testicular tissue and accessory glands of the boar are bacteria-free, and therefore bacterial contamination of the ejaculate occurs during the semen collection process (Martin Rillo *et al.*, 1998). An antibiotic needs to be added to the diluent since its components (glucose) and the temperature at which semen doses are stored (15-16ºC) promote the growth of most Gram negative bacteria (including *Escherichia Coli* and some *Salmonella* and *Pseudomonas* species).

Bacterial contamination mainly leads to a series of alterations including diminished sperm motility, sperm agglutination, or «clumping», an increased proportion of altered acrosomes and pH lowering to acidic levels (5.7-6.4) (Althouse *et al.*, 2000). These factors all shorten the length of time semen doses can be preserved. Thus, adding an antibiotic at the appropriate concentration improves sperm survival and, in turn, improves fertility results (reviewed in Colenbrander *et al.*, 1993). Further, the appropriate use of antibiotics could even represent a huge advance, if the conditions of hygiene in which semen is collected and the doses processed were also improved (Almond and Poolperm, 1996).

Penicillin plus streptomycin (1 g L^{-1}) was initially the combination most frequently used. Subsequent to this,

aminoglycosides were —and still are— successfully used including gentamicin, neomycin and kanamycin at concentrations around 200 mg L^{-1}. Most recently, new generation antibiotics (ceftiofur, apramycin, etc.) are being used, though no conclusive results are available yet.

At the legislation level, there are two reference organisations: the Office International des Epizooties (OIE) and the European Union (EU).

In its International Animal Health Code (2002), the OIE regulates the criteria to be applied to semen diluents. This norm basically recommends that if a diluent contains an ingredient such as milk, egg yolk or other animal protein, these should be pathogen-free or sterilised. The addition of antibiotics is permitted, provided they are declared in the corresponding international veterinary certificates.

In the EU setting, Directive 90/429/CEE (OJ, 1990), which regulates health policies applied to exchanges between member countries and the import of boar sperm, stipulates the use of a combination of antibiotics that should be efficient particularly against leptospirochaetes and mycoplasmas. Its concentration should at least have an effect equivalent to the following: 500 IU ml^{-1} streptomycin, 500 IU ml^{-1} penicillin, 150 mg ml^{-1} lincomycin or 300 mg ml^{-1} spectinomycin. This norm also indicates that immediately after adding the antibiotics, the diluted semen should be kept at a temperature of at least 15ºC for a minimum of 45 min.

Fertility tests

The efficiency of semen extenders in reproduction terms has been extensively evaluated. First, we should consider that the relationship between semen quality (which the diluent preserves) and resultant fertility is not direct (Gadea *et al.*, 1998; Gadea, 2001). Moreover, studies assessing boar semen diluents have been conducted in very different experimental conditions (animal species, environmental conditions, number of inseminations, number of spermatozoa per dose, time of AI, etc.). Thus, comparisons need to be undertaken with particular caution and the conclusions to be drawn are of a general nature and applicable only to the majority of cases.

Preservation time

Several investigations have analysed the effect of storing boar semen in different diluents on fertility af-

Table 3. Fertility and litter size assessed after insemination with semen preserved in BTS

Hours of preservation	Fertility (%)	TNB	NBA
4-14	67.8[a]	11.96[a]	10.94[a]
28-38	69.8[a]	11.73[b]	10.73[b]
52-62	64.6[b]	11.61[b]	10.64[b]

[a,b]: different letters in the same column indicate a significant difference at $p<0.05$. NBA: number of piglets born alive; TNB: total number of piglets born. *Source:* Hofmo (1991).

ter AI. Thus, the diluent BTS (Hofmo, 1991) gives rise to a significant reduction in fertility when the diluted semen is stored for 48 h, while the total number of piglets born (TNB) and the number of piglets born alive (NBA) significantly decreases after 24 h of storage (Table 3). Similar results were obtained by Alexopoulos *et al.* (1996) who noted reduced fertility when the semen was stored for more than 72 h in BTS.

In contrast, Martínez *et al.* (1986) found that diluent MR-A was able to maintain the same fertility rate and number of live births using semen stored for up to 5 days (Table 4). In a subsequent study, however, it was concluded that fertility significantly decreases when semen is preserved in this medium for 7-8 days (84 *vs.* 67.3%) while litter size is not appreciably affected (11.1 *vs* 10.7) (Lyczynski and Kolat, 1996).

Comparing diluents

Many studies have centred on evaluating the new diluents with respect to well known extenders. Of all the available data, we will discuss the most significant findings.

Although a higher sperm survival rate (measured as motility) has been observed with the long-term extenders (MR-A and Androhep) compared to the short-

Table 4. Fertility and litter size assessed after insemination with semen preserved in MR-A

No. days' storage	No. services	Fertility (%)	TNB
0-1	136	84.5	8.9
1-2	145	82.7	9.2
2-3	170	86.4	9.3
3-4	104	81.7	8.9
4-5	99	83.8	9.2

TNB: total number of piglets born. *Source:* Martínez *et al.* (1986).

Table 5. Fertility and litter size following insemination with semen preserved in Kiev or MR-A

Days	Kiev			MR-A		
	N	Fertility (%)	TNB	N	Fertility (%)	TNB
1	20,121	85.0	11.6	12,556	85.6	11.9
2	14,968	83.7	11.4	13,139	84.7	11.8
3	2,968	82.2	10.9	9,110	82.6	11.5
4	2,94	82.3	10.5	4,775	82.0	11.2
5				1,747	80.4	10.8
6				706	81.5	11.2
7				183	81.4	10.4
Total	38,351	84.3	11.5	42,216	84.0	11.6

Source: Ratto and Jokinen (1990).

term diluent Kiev (Korniewicz *et al.*, 1996), this difference was not significant when Androhep was compared to BTS (Waberski *et al.*, 1994a). Recently, Huo *et al.* (2002) performed an interesting study on the quality of semen diluted in different media (Androhep, Zorlesco, BTS and Kiev) and stored for up to 15 days. Their findings indicated that sperm viability and mitochondrial activity exceeded 50% after 13 days of storage in long-term diluents (Androhep and Zorlesco).

The Kiev extender provided improved fertility results over those achieved with Beltsville liquid I (BL1) after 1 (74.5 *vs.* 64.7%) or 3 days (65.9 *vs* 60.5%) of storage (Johnson *et al.*, 1982). In subsequent evaluations of short-term diluents, BTS was found to be more efficient than Kiev, Zorlesco and Modena in terms of fertility (Aalbers *et al.*, 1983; Blichfeldt *et al.*, 1988).

When we compared short-term diluents to those of long duration, no significant differences in fertility or litter size were generally noted in the first 3-4 days of storage, although the long-term extenders could be used for up to 7 days (Table 5). This is reflected by the results reported by Ratto and Jokinen (1990) when Kiev was compared to MR-A. Similar findings were

described by Johnson *et al.* (1988), when they compared BTS to MR-A and Modena. These authors detected no significant differences between BTS and MR-A when used for up to 4 days of storage, but fertility rates and litter sizes were significantly improved over those recorded for Modena (Table 6) after AI of multiparous sows. However, these differences were diminished when the diluted semen was used to inseminate gilts, with no differences observed in litter size among the three diluents assessed. Similarly, Hofmo *et al.* (1998) noted no significant differences related to the use of BTS to preserve semen for 2-3 days compared to MR-A for 4-5 days.

Weitze (1990) compared the litter sizes obtained using semen stored for 3 or 5 days in Androhep, BW25 or Kiev (Table 7). No differences were recorded in terms of fertility. The study involved simple insemination using 2×10^9 spermatozoa per dose and the authors reported reduced litter sizes as the storage time increased. Later, when Waberski *et al.* (1994a) compared the media Kiev and Androhep used for storing semen for 2 to 5 days, they observed similar fertility results while the litter size was greater for Androhep after the fourth day of storage.

Table 6. Fertility and litter size after AI using semen preserved in BTS, Modena or MR-A

	Multiparous sows			Gilts		
	BTS	Modena	MR-A	BTS	Modena	MR-A
N	721	700	720	103	117	91
% Fertility	79.3[a]	50.4[b]	77.6[a]	73.5[a]	50.2[b]	64.1[ab]
TNB	11.4[a]	10.0[b]	11.1[a]	9.5[a]	8.5[a]	9.6[a]
NBA	10.7[a]	9.4[b]	10.5[a]	8.9[a]	7.8[a]	9.0[a]

[a,b]: different letters in the same column indicate a significant difference at p<0.004. NBA: number of piglets born alive; TNB: total number of piglets born. *Source:* Johnson *et al.* (1988).

Table 7. Fertility and litter size after AI using semen preserved for 3 or 5 days in Androhep, BW25 or Kiev

	N		% Fertility		TNB	
	3 days	5 days	3 days	5 days	3 days	5 days
Androhep	351	316	76.9	73.1	10.9[a]	10.4[b]
BW25	361	357	76.7	67.2	10.8	10.2
Kiev	404	350	75.1	71.1	10.7[a]	10.0[b]

[a,b]: different letters in the same row indicate a significant difference at $p < 0.05$; TNB: total number of piglets born. *Source:* Weitze (1990).

Finally, other studies have compared long-term diluents among themselves. Laforest and Allard (1996) in Canada compared the diluents MRA, BTS, Modena and Androhep and found no significant differences in fertility neither among the diluents nor according to the time of preservation (1-2 days *vs* 3-4 days). Only Modena was related to a discretely reduced total number of piglets born (TNB) after 3-4 days of storage. Kuster and Althouse (1999) analysed the use of Androhep and X-Cell, observing similar fertility results for the two diluents and the different number of days of semen storage (up until 5 days). However, Androhep was associated with reduced fertility on the sixth day of preservation and with a reduced litter size on the fifth day (Table 8).

Future perspectives

After almost a century of artificial insemination in swine, knowledge about the preservation of boar spermatozoa is still very limited and demands that future studies take the following directions:

a)   Up until now, the design of new diluents has been based on an empirical model. In the future we will need to gain more insight into the sperm cell and its metabolism, and new models are needed to evaluate and optimise the diluent's components (Pettit *et al.*, 1999). Several additives are currently being evaluated that could affect sperm viability (e.g., alkyl-glycerol by Cheminade *et al.*, 2002), protect against cold shock (Zeng and Terada, 2001) or improve sperm transport, synchrony with ovulation (Waberski *et al.*, 1994b) and the fertilisation process (reviewed in Waberski, 1997; Kemp and Soede, 1997).

b)   The possibility of preserving semen at temperatures below 15ºC would help reduce sperm metabolic activity and protect against the detrimental effects of microbial contamination. This type of investigation should address the cold shock problem. Several research teams are presently engaged in evaluating the effects of temperature on sperm survival (Althouse *et al.*,1998; Paulenz *et al.*, 2000) and especially on fertility results. Althouse *et al.* (1998) reported similar results for fertility (93 *vs* 95%) and litter size (TNB, 11.58 *vs* 11.61; NBA, 10.68 *vs* 10.63) related to the use of semen stored for 60 h at 12 or 17ºC. Foote (2002b) has recently published promising results after preserving boar semen at 5ºC in media containing egg yolk.

c)   Diluents also need to be adapted to individual differences among animals. Levis (2000) suggests the revision of several features of AI that have not been extensively explored including individual differences, since not all males interact in the same way with the diluents used (Weitze, 1990). Different breed-diluent interactions have also been described (Richter and Claus, 1990).

Table 8. Fertility and litter size after AI using semen preserved in Androhep or X-Cell

Days	Androhep			X-Cell		
	N	Fertility (%)	TNB	N	Fertility (%)	TNB
2-3	170	85.9	10.3	172	86.1	10.2
3-4	164	86.6	9.2	151	84.1	10.0
4-5	188	85.1	9.0*	183	86.3	10.4*
5-6	201	78.6*	9.4	202	85.6*	9.7

* $p < 0.01$; NBA: number of piglets born alive; TNB: total number of piglets born. *Source:* Kuster and Althouse (1999).

d)   The use of new techniques such as deep intrauterine insemination, in which the number of spermatazoa per dose and insemination volume is reduced, could require new preservation conditions and accordingly, the diluent will need to be better assessed for this purpose (reviewed in Rath, 2002).

Practical conclusions

The choice of diluent should depend on its proposed use. When it is planned that the time from semen collection to use will be less than 3 days, the most rational choice would be to use a short-term diluent (of the BTS or Kiev type). The results of previous studies suggest no appreciable effects on semen quality or resultant fertility rates. Moreover, this type of diluent is less expensive and results are similar to those achieved with a long-term extender.

When the semen dose needs to be preserved for more than 4 days before insemination (in the case of long distances, health inspection of the semen, etc.) a long-term semen extender should be used and the sperm concentration of the dose should be increased. This precaution will compensate for any loss of sperm viability through ageing.

Whether a short- or long-term diluent is used, it should always be borne in mind that there are many factors than can affect the fertility results obtained. Thus, every effort possible should be made to optimise essential procedures such as oestrus diagnosis and selecting and processing the semen doses to ensure the best semen quality possible. The choice of diluent should always be aimed at optimising subsequent fertility rates and litter sizes in the particular conditions of each pig farm, since economic repercussions on production can be devastating.

References

AALBERS J.G., RADEAMAKER J.H.M., GROTEN H.J.G., JOHNSON L.A., 1983. Fecundity of boar semen stored in BTS, Kiev, Zorlesco and Modena extenders under field conditions. J Anim Sci 57 (Suppl. 1), 314-315.

ALEXOPOULOS C., BOSCOS C., SARATSIS PH., SAOULIDIS C., KYRIAKIS S., 1996. The effect of storage time and number of spermatozoa per insemination dose on semen characteristics and fertilizing capacity of boar semen diluted with Beltsville Thaw Solution (BTS) extender. Anim Sci 62, 599-604.

ALMOND G., POOLPERM P., 1996. Semen contamination and choosing antibiotics. Proceedings of the North Carolina Healthy Hogs Seminar. Available at: http://mark.asci.ncsu.edu/HealthyHogs/book1996/book96_5.htm

ALTHOUSE G.C., 1997. Comparison of currently used semen extenders in the swine industry. Compend Cont Educ Pract Vet 19, 777-782.

ALTHOUSE G.C., KUSTER C.E., CLARK S.G., WEISIGER R.M., 2000. Field investigations of bacterial contaminants and their effects on extended porcine semen. Theriogenology 53, 1167-1176.

ALTHOUSE G.C., WILSON M.E., KUSTER C., PARSLEY M., 1998. Characterization of lower temperature storage limitations of fresh-extended porcine semen. Theriogenology. 50, 535-543.

ALVAREZ J.G., STOREY B.T., 1982. Spontaneous lipid peroxidation in rabbit epididymal spermatozoa: its effect on sperm motility. Biol Reprod 27, 1102-1108.

BLICHFELDT T., ALMID T., STAVNE S.E., 1988. Liquid preservation of boar semen in Kiev or BTS. A field comparison. 11th ICAR. Dublin, 3, 231.

CHEMINADE C., GAUTIER V., HICHAMI A., ALLAUME P., LE LANNOU D., LEGRAND A.B., 2002. 1-O-alkylglycerols improve boar sperm motility and fertility. Biol Reprod 66, 421-8.

CHENG T.T.K., 1985. In vitro fertilization of farm animal oocytes. PhD Thesis, Council for National Academic Awards. UK.

COLENBRANDER B., FEITSMA H., GROOTEN H.J., 1993. Optimizing semen production for artificial insemination in swine. J. Reprod. Fert. Supp. 48, 207-215.

CRABO B.G., 1990. Preservation of boar semen: a worldwide perspective. Reprod Dom Anim Suppl 1, 3-9.

DU MESNIL DU BUISSON F., DAUZIER L., 1959. Improvement of practical use of preservation techniques for boar semen by saturation of the diluent with carbondioxide. Ann Zootech Suppl 8, 81-96.

FLOWERS W.L., ESBENSHADE K.L., 1993. Optimizing management of natural and artificial matings in swine. J Reprod Fert Supp 48, 217-228.

FOOTE R.H., 2002a. The history of artificial insemination: Selected notes and notables. J. Anim. Sci. Biography and History Series, 1-10.

FOOTE R.H., 2002b. Within-herd use of boar semen at 5 degrees C, with a note on electronic monitoring of oestrus. Reprod Domest Anim 37, 61-63.

FRASER L., GORSZCZARUK K., STRZEZEK J., 2001. Relationship between motility and membrane integrity of boar spermatozoa in media varying in osmolality. Reprod Domest Anim 36, 325-329.

GADEA J., MATAS C., LUCAS X., 1998. Prediction of porcine semen fertility by homologous in vitro penetration (hIVP) assay. Anim Reprod Sci 54, 95-108.

GADEA J., SELLÉS E., TOMÁS P., RUIZ S., 2001. El valor del análisis seminal porcino en las condiciones de explotación comercial. ITEA 22, 829-831.

GILMORE J.A., DU J., TAO J., PETER A.T., CRITSER J.K., 1996. Osmotic properties of boar spermatozoa and

their relevance to cryopreservation. J Reprod Fertil 107, 87-95.

GOTTARDI L., BRUNEL L., ZANELLI L., 1980. New dilution media for artificial insemination in the pig. 9th ICAR. Madrid. 5, 49-53

HARRISON R.A.P., DOTT H.M., FOSTER G.C., 1978. Effect of ionic strength, serum albumin and other macromolecules on the maintenance of motility and the surface of mammalian spermatozoa in a simple medium. J Reprod Fert 52, 65–73.

HOFMO P.O., 1991. Commercial swine artificial insemination with liquid boar semen in Norway. Reprod. Domest. Anim. Suppl 1, 317-320.

HOFMO P.O., KOMMISRUD E., SEHESTED E., 1998. A field trial comparing the fertility of fresh semen stored in BTS for 2-3 days and MR-A for 4-5 days. 15th IPVS Congress, 3, p 64.

HUO L.J., MA X.H., YANG Z.M., 2002. Assessment of sperm viability, mitochondrial activity, capacitation and acrosome intactness in extended boar semen during long-term storage. Theriogenology 58, 1349-1360.

ITO T., NIWA T., KUDO A., 1948. Studies on artificial insemination in swine. Zootech Exp Sta Res Bull 55, 1-74.

IVANOW E.I., 1907. De la fécondation artificielle chez les mammifères. Arch Sci Biol 12, 377-511.

IVANOW E.I., 1922. On the use of artificial insemination for zootechnical purposes in Russia. J Agric Sci 12, 244-256.

JOHNSON L.A., AALBERS J.G., GROOTE H.J.G., 1988. Artificial insemination of swine: Fecundity of boar semen stored in Beltsville Thawing Solution (BTS), modified modena (MM), or MR-A and inseminated on one, three and four days after collection. Zuchthyg 23, 49-55.

JOHNSON L.A., AALBERS J.G., WILLEMS C.M., RADEMAKER J.H., REXROAD C.E. JR., 1982. Use of boar spermatozoa for artificial insemination. III. Fecundity of boar spermatozoa stored in Beltsville liquid and Kiev extender for three days at 18ºC. J Anim Sci 54, 132-136.

JOHNSON L.A., WEITZE K.F., FISER P., MAXWELL W.M.C., 2000. Storage of boar semen. Anim Reprod Sci 62, 143 –172.

KEMP B., SOEDE N.M., 1997. Consequences of variation in interval from insemination to ovulation on fertilization in pigs. J Reprod Fertil Suppl 52, 79-89.

KORNIEWICZ D., SZCZESNIAK-FABIANCZYK B., SMORAG Z., 1996. The survival rate and fertilizing capacity of boar semen diluted with different diluents. Reprod Domest Anim 31, 273-274.

KUSTER C.E., ALTHOUSE G.C., 1999. The fecundity of porcine semen stored for 2 to 6 days in Androhep and X-Cell extenders. Theriogenology 52, 365-376.

LAFOREST J.P., ALLARD D., 1996. Comparison of four extenders for long-term storage of fresh boar semen. Reprod Domest Anim 31, 275-276.

LEVIS D.G., 2000. Liquid boar semen production: Current extender technology and where do we go from here! In: Semen boar preservation IV (L.A. Johnson and H.D. Guthrie, eds.). Allen Press, Inc. Lawrence, KS, pp. 121-128.

LEWIS L.L., 1911. The viability of reproductive cells. Agri. Exp. Sta. Bull. No. 96. Oklahoma Agriculture and Mechanical College. Stillwater. OK

LYCZYNSKI A., KOLAT K., 1996. Boar semen preservation in MR-A diluent. Reprod Dom Anim 31, 271-272.

MARTÍN RILLO S., 1984. How AI is progressing in Spain. Pig Intern (May) 24-28.

MARTÍN RILLO S., SHOKOUHI V., GARCIA BOIX E., HERNANDEZ GIL R., ROMERO L., 1998. Contamination of semen doses and its possible relationship with the bacterial flora of the prepuce. 15th IPVS Congress. 3, p 60.

MARTÍNEZ E., RUIZ S., SEBASTIÁN J., SÁNCHEZ R., GARCÍA C., MARTÍN S., 1986. Factores que afectan a la inseminación artificial porcina. An Vet Murcia 2, 115-120.

MCKENZIE E.E., 1931. A method for collection boar semen. J Am Vet Assoc 78 (News series 31), 244-246.

MILOVANOW V.K., 1938. Artificial insemination in domestic animals (published in Russian). Seljhozgiz, Moscu.

MORETTI J., 1981. (cited by Johnson L.A., Aalberts J.G., 1984). Artificial insemination of swine: fertility using several liquid semen diluents. 8th IPVS Congress Ghent, Belgium, p 293.

NEWTH M.S., LEVIS D.G., 1999. Change in pH boar of semen extenders. 1999 Nebraska Swine Report, pp 3-6.

OFFICE INTERNATIONAL DES EPIZOOTIES (OIE), 2002. International Animal Health Code. 11th Edition.

OJ, 1990. Directive 90/429/CEE of the Council of June 26, 1990. Official Journal of the European Union L 224 18/08/1990, p.62

PAULENZ H., KOMMISRUD E., HOFMO P.O., 2000. Effect of long-term storage at different temperatures on the quality of liquid boar semen. Reprod Dom Anim 35, 83-85.

PETTIT M.J., PLANTE C., POLLARD J.W. 2000. Long term fresh extender development by simplex optimisation. IV Int. Conf. Boar Semen Preservation. Beltsville. p 16.

PIG INTERNATIONAL, 1998. How big is artificial insemination now? Pig Intern 28(2), 9-10.

PLISKO N.T., 1965. Method of prolonging the viability and fertilizing capacity of boar spermatozoa. Svinovodstvo 9, 37-41.

POLGE C., 1956. Artificial insemination of pigs. Vet Rec 68, 62-76.

PURSEL V.G., JOHNSON L.A. 1975. Freezing of boar spermatozoa: fertilizing capacity with concentrated semen and a new thawing procedure. J Anim Sci 40, 99-102.

PURSEL V.G., JOHNSON L.A., SCHULMAN L.L., 1973a. Effect of dilution, seminal plasma and incubation period on cold shock susceptibility of boar spermatozoa. J Anim Sci 37, 528-531.

PURSEL V.G., JOHNSON L.A., SCHULMAN L.L., 1973b. Fertilizing capacity of boar semen stored at 15ºC. J Anim Sci 37, 532-535.

RATH D., 2002. Low dose insemination in the sow - a review. Reprod Domest Anim 37, 201-205.

RATTO J., JOKINEN L., 1990. Reports about number of swine inseminations and farrowing results in Finland 1989, comparison between two diluents EDTA and MR-A. Reprod Dom Anim Suppl 1, 365-368.

REED H.C.B., 1985. Current use of frozen boar semen. Future need of frozen boar semen. Deep freezing of boar semen. Ed Johnson LA, Larsson K. Swedish University of Agricultural Sciences, pp 225-237.

REED H.C.B., 1990. Commercial requirements for an effective fresh semen diluent. Reprod Dom Anim Suppl 1, 255-270.

REED H.C.B., CURNOCK R.M., 1990. Comparison of three liquid semen diluents in a national semen delivery service in Great Britain. Reprod Dom Anim Suppl 1, 369-373.

REVELL S.G., GLOSSOP C.E., 1989. A long-time ambient temperature diluent for boar semen. Anim Prod 48, 579-584.

RIGAU T., PIEDRAFITA J., REVERTER J., CANAL M., RODRÍGUEZ-GIL J.E., 1996. The rate of L-lactate production: a feasible parameter for the fresh diluted boar semen quality analysis. Anim Reprod Sci 43, 161-172.

RITCHER L.U., CLAUS H., 1990. Breed specific diluters as an indication for better semen preservation. Reprod Dom Anim Suppl 1, 398.

RODIN I.M., LIPATOV V.I., 1935. Artificial insemination of pigs. Anim Breed Abstr 4, 205.

ROUCO A., MUÑOZ A., 1998. Economic weights determination of zootechnical parameters in pig production. Proc. 15th IPVS Congress, Birmingham, 3, p 8.

SEE M.T., 1996. A cost comparison of AI and natural service. Animal Science Facts. No. ANS-96-809S. Agricultural Publications. NC State University.

WABERSKI D., 1997. Effects of semen components on ovulation and fertilization. J Reprod Fertil Suppl 52, 105-109.

WABERSKI D., MEDING S., DIRKSEN G., 1994a. Fertility of long-term stored boar semen: Influence of extenders (Androhep and Kiev), storage time and plasma droplets in the semen. Anim Reprod Sci 36, 145-151.

WABERSKI D., WEITZE K.F., LIETMANN C., 1994b. The initial fertilizing capacity of long-term stored liquid boar semen following pre and postovulatory insemination. Theriogenology 41, 1367-1377.

WABERSKI D., WEITZE K.F., RATH D., SALLMANN H.P., 1989. Effect of bovine serum albumin and zwitterionic buffers on stored liquid boar semen. Zuchthygiene 24, 128-133.

WEITZE K.F., 1990. Long- term storage of extended boar semen. Reprod Dom Anim Suppl 1, 231-253.

WESTENDORF P., RICHTER L., TREU H., 1975. Deep freezing of boar sperma. Laboratory and insemination results using the Hülsenberger paillete method. (in German). Dtsch Tierarztl Wochenschr 82, 261-267.

WHITE I.G., 1993. Lipids and calcium uptake of sperm in relation to cold shock and preservation: a review. Reprod Fertil Dev 5, 639-658.

ZENG W.X., TERADA T., 2001. Protection of boar spermatozoa from cold shock damage by 2-hydroxypropyl-beta-cyclodextrin. Theriogenology 55, 615-27.