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Sperm Morphology Assessment—Historical Perspectives and Current Opinions

Andrology Lab Corner

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Although it is now widely recognized that sperm morphology is the semen characteristic most correlated with fertility and, in particular, fertilizing ability in vitro, many workers remain confused about the origins and specific features of the various criteria and classification schemes used to assess human sperm morphology. The purpose of this article is to review the origins and history of the two major, and often apparently opposing, scoring systems: those of the World Health Organization (WHO) and the Tygerberg Strict Criteria. The similarities and differences between these two approaches will be discussed and their application compared.

What Is a Normal Human Spermatozoon?

In the earliest reports on human sperm morphology, the ''normal'' or ''typical'' spermatozoon was described as the modal form, ie, the shape that occurred most often. Briefly, these authors focused on the sperm head, describing it as being oval with a smooth contour and divided into anterior ''acrosomal'' and posterior ''postacrosomal'' regions, adding that there should be a single tail with a symmetric axial attachment at the sperm neck, where the proximal part of the tail was thickened in the midpiece region. Regardless of any other considerations, such as whether morphological abnormalities were prioritized in order of head, neck, midpiece, or tail regions or whether defects were assessed multiparametrically, one difference between the past and present is immediately obvious: if the cutoff (or reference value) for normality is now more than 14%, then clearly the normal form of today cannot be the modal form. Further, it is clear that the average percentage of morphologically normal spermatozoa being reported today is considerably lower than that described 30, or even 10, years ago (Figure 1). Although most of the decline in the percentage of normal forms, especially in the earlier years, can probably be attributed to stricter evaluation, a real decline in the actual prevalence of morphologically normal spermatozoa cannot be ignored (Menkveld et al, 1986, 1997a; Menkveld, 1987). The big question, which will probably be difficult to answer, is how much of the decline, particularly in the earlier years, should be attributed to each of the two factors. Certainly, changes in assessment standards and interpretation of what sperm morphology results actually mean have lead to great confusion for scientists and clinicians.

Before discussing how normal spermatozoa should be defined, another troubling matter must be considered—namely, the relationship between sperm morphological normality and fertilizing ability. It is not uncommon for laboratory andrologists to be asked, ''if this man has so very few [no] morphologically normal sperm, how can he possibly achieve fertilization?'' Such questions typify the general lack of understanding of just what sperm morphology assessments are, what can be inferred from them, and the mechanics and cell biology of fertilization. In addition, this confusion has been compounded by the fact that many persons evaluating sperm morphology become overstrict; in such circumstances, the predictive value can be lost and we find ourselves on the other extreme of the situation, compared with the early ''lenient'' criteria (see below). Such changes over time demonstrate the importance of internal quality control, which is essential even for the most experienced observers.

First, there is *no* a priori equivalence between sperm morphological normality and fertilizing ability. Not all normal spermatozoa are able to fertilize, and, although some abnormal spermatozoa (eg, acrosomeless forms) certainly cannot fertilize except by intracytoplasmic sperm injection (ICSI), others can. For example, a spermatozoon might have an apparently perfect morphology when examined by use of light microscopy, but if the man has Kartagener's syndrome (immotile cilia syndrome),

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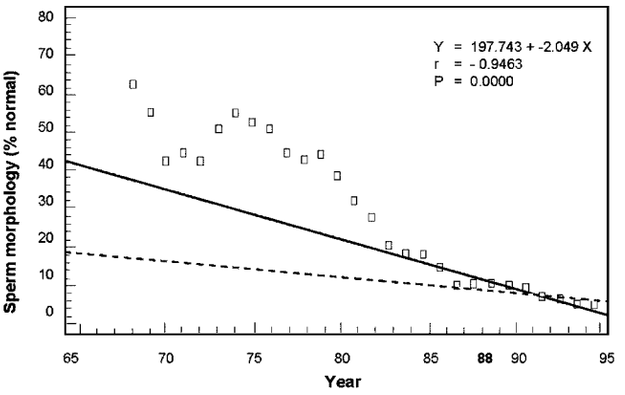

Figure 1. Scatter diagram of mean percentage of morphologically normal spermatozoa over a period of 28 years (1969 to 1995) at Tygerberg Hospital. Notations in the figure area represent data over the whole period of 28 years. The solid line represents the decline in actual mean values of morphologically normal spermatozoa over the 8-year period of 1988 to 1995, from 12.4% to 7.8% (difference = 4.6 %). The broken line represents the decline due to a more stringent approach in the evaluation of the sperm morphology over that time, based on re-evaluation of a cohort of 1988 samples in 1996 with mean values of morphologically normal spermatozoa of 12.4% and 9.5% respectively, ie, a difference of 2.9%. The residual difference of 1.7% is attributed to other (environmental) factors. For more detail, see Menkveld et al (1997a).

with all his spermatozoa being immotile, then he is functionally sterile unless ICSI is employed. Conversely, a diploid spermatozoon is certainly morphologically abnormal, yet cases of androgenetic triploidy can arise from diplospermy, ie, fertilization by a diploid spermatozoon, not just from dispermy.

In reality, a sperm morphology assessment as part of a semen analysis is a visual appraisal of the shape and conformation of spermatozoa prepared as air-dried, fixed, and stained smears examined with the resolving power of a light microscope. When scoring these preparations, we are evaluating the appearance of the spermatozoa and comparing it to expectations that have been derived (almost always by others) from studies of other men's spermatozoa, in which morphology was correlated with an endpoint of interest, such as spontaneous in vivo conception (eg, men whose wives are in the first trimester of pregnancy, the generally accepted best definition of a fertile man) or fertilization in vitro (perforce based on populations of infertile couples). Consequently, the evaluation is indirect and, like all interpretations derived from population studies, subject to insensitivity or lack of specificity when applied to individual cases. Furthermore, sperm morphology is being evaluated independently of sperm motility, kinematics, vitality, and every other physiological process that is involved in the complex sequence of events required for gamete approximation and interaction that lead to fertilization under natural conditions. Hence, it is totally unrealistic and naive of anyone to expect that a morphology assessment—no matter how carefully performed—will be either 100% correct or provide the whole answer as to a man's in vivo fecundity or his in vitro fertilizing potential. We are merely obtaining a morphological indicator or index as to the likelihood that a given man will, or will not, be able to achieve the endpoint of interest; a sperm morphology result is not an absolute truth.

How Are ''Normal'' Spermatozoa Defined?

In the early ''modern'' studies by workers such as John MacLeod (eg, MacLeod, 1964, 1970), the ''oval form'' was considered to reflect morphological normality, stemming from earlier work by many investigators, including Moench, Hotchkiss, Williams, and Hammen, who provided many wonderful drawings and descriptions of human sperm morphology (see Hammen, 1944). Morphometric studies by van Duijn et al (1972) provided accurate dimensions for human sperm heads and their differences between normal and subfertile men, although the issue was confused by poor textbook illustrations that, although defining the oval form to be the modal shape for the human sperm head, showed it as a clearly abnormal shape (eg, Hafez, 1980). The original greater than 60% cutoff for normal sperm morphology was established in this era, when assessments followed the ''liberal'' view—indeed, Macleod and Gold (1951) stated that ''we are not prepared at this time to classify any but the most distorted forms as truly abnormal.''

This descriptive approach remained the generally accepted approach for defining normality until the late 1970s and the 1980s, when careful studies on sperm functional ability and selection in vitro and in vivo were reported (Fredricsson and Björk, 1977; Mortimer et al, 1982; Pretorius et al, 1984; Gonzalès and Jézéquel, 1985; Ragni et al, 1985; Katz et al, 1990). However, it should not be forgotten that several earlier authors had remarked on the selection of human spermatozoa in postcoital cervical mucus (eg, Cary and Hotchkiss, 1934; Kremer, 1968; MacLeod, 1970), although they did not consider it in defining morphological normality.

The present authors met for the first time in Professeur Georges David's laboratory in Kremlin-Bicêtre (near Paris, France) in July 1981. David Mortimer (DM) was doing postdoctoral research on sperm kinematics, and Roelof Menkveld (RM) was visiting various laboratories around Europe that had been instrumental in developing or applying classification schemes for human sperm morphology (see below). RM had been elaborating the concepts that were crystallizing into the origins of what is now known as the Tygerberg Strict Criteria, and DM was preparing for publication much of his PhD and early postdoctoral work from Edinburgh on the selection of human

spermatozoa. The common interest in looking at postcoital or Kremer tests to determine the selective effect of mucus penetration on spermatozoa led to an enduring friendship. Differences in approach, ie, the search for ''perfect'' spermatozoa (RM), compared with the focus on identifying defects (DM), have been seen by many as mutually exclusive, although both lead ultimately to remarkably similar conclusions on the biological importance of sperm morphology. Indeed, as knowledge of the relationship between sperm morphology and fertilizing ability has increased, RM has expanded the Tygerberg scheme to include other concepts such as the ''acrosome index'' (Menkveld et al, 1996; see below).

Another perspective mutually shared by the authors is the interpretation of studies on sperm morphological selection and how these relate to physiology. In particular, we believe that selection takes place not only at the level of cervical mucus penetration, but also at sperm penetration through the cumulus oophorus and corona radiata, and at the level of binding to the zona pellucida and subsequent sperm penetration through the zona before binding to the oolemma. However, just because selection might occur in a particular location does not mean that the structure or environment itself is actually performing that selection of spermatozoa. For example, sperm migration through the cumulus is clearly dependent on sperm motility—in particular, hyperactivated motility—and, with the reported association between sperm morphological normality and motile ability (see below), any apparent morphological selection does not need to specifically occur on the basis of morphological features of the spermatozoa: it can be indirect, via motility (and perhaps in conjunction with other physiological phenomena). An extreme example of this is the recent paper by Van Dyk et al (2000) that concludes, ''[t]his study suggests that the human zona pellucida has the capacity to select against aneuploid spermatozoa by an as yet unknown mechanism.'' For such a mechanism to exist, the zona pellucida needs both detector and effector capabilities—ie, it must be able to detect differences in the genetic content of spermatozoa that attach to it and then be able to bind genetically normal spermatozoa with higher affinity (and/or reject those with genetic abnormalities). Such direct mechanisms are certainly very difficult to postulate, but, if one accepts fundamental relationships between spermiogenesis and morphologically normal spermatozoa and that such spermatozoa have better fertilizing ability, then the concept that, in reality, the spermatozoa are actually selecting themselves on the basis of their intrinsic functional ability becomes not only attractive but biologically reasonable. Supporting evidence for such an opinion includes 1) morphologically normal spermatozoa seem to have better motility than abnormal ones (eg, Katz et al, 1982, 1990; Morales et al, 1988); 2) more morphologically normal spermatozoa are found bound to the zona pellucida than abnormal ones in vitro (Menkveld et al, 1991a; Liu and Baker, 1992, 1994; Garrett et al, 1997); and 3) morphologically abnormal spermatozoa have a higher prevalence of chromosomal abnormalities (Lee et al, 1996; Kishikawa et al 1999). Basically, it can therefore be hypothesized that structures such as cervical mucus, the cumulus oophorus, and the zona pellucida act as barriers where sperm selection can take place but do not constitute the selective process per se.

However, DM also holds the further belief that the selective ability of the zona pellucida to bind only spermatozoa with certain morphological forms during in vitro binding tests is likely to reflect an extreme relationship because of the inherent difference between sperm binding to a naked (hemi)zona pellucida and the physiological situation that exists both in the ampulla of the fallopian tube or during in vitro fertilization (IVF), when spermatozoa encounter oocytes that are enclosed by the cumulus-corona complex (Drobnis et al, 1988). In other words, the geometry of a fertilizing spermatozoon's head contacting the zona pellucida would be very different when the spermatozoon is held in place by virtue of its being enclosed within the cumulus, usually with the head trapped between cells of the corona radiata. For DM, this might be part of the explanation why fertilization can occur in the absence of any strictly ''normal'' spermatozoa, precise head geometry being not so vitally important as during the hemizona assay. Nonetheless, this does not fully account for the findings of Krzanowska and Lorenc (1983) that, in the mouse, the proportion of abnormal sperm heads seen inside the ooplasm was significantly lower in cumulus-intact than in cumulus-free oocytes and that most severely abnormal forms were not found in intact oocytes.

Regardless of such slight differences of opinion, we now look at sperm morphology with a very different perspective, compared with the historical workers whose insights were, perforce, purely descriptive. We define normality in light of knowing which spermatozoa might attain the site of fertilization in vivo, which are best able to bind to the zona pellucida, and which men achieve successful fertilization at IVF. Certainly we have access to microscopes with far better optics than those typically seen in pathology laboratories, especially 50 or 60 years ago. However, we have also created a strong negative influence in our work as well—the obsession with quick staining methods that are seen as ways of saving time (and hence money) without true regard to using the best available techniques.

Staining Methods for Human Spermatozoa

In these authors' opinion, Papanicolaou staining technique provides for the best sperm morphology assess-

ment. MacLeod thanked George Papanicolaou for his support and friendship (MacLeod, 1964), and Eliasson has always recommended that sperm smears should be stained by the Papanicolaou method (Eliasson, 1971, 1981). Apart from an aberration in the second edition of its manual (World Health Organization, 1987), the WHO has always recommended Papanicolaou staining, and the present authors have both stated that it gives the best differentiation for routine application (Mortimer, 1985, 1994; Menkveld et al, 1990). In several laboratories, DM has investigated the use of alternative, faster staining methods such as Testsimplets (Boehringer, Mannheim, Germany; eg, Schirren et al, 1977), Sangodiff (Merck, Darmstadt, Germany), Diff-Quik (Dade Diagnostics, Miami, Fla), Spermac (Oettlé, 1986), or Shorr staining (David et al, 1975), but in all cases they were found to give poorer quality preparations. Actually, in each case the technical staff who performed the morphology assessments themselves stated that they preferred to spend the extra time staining smears by the Papanicolaou method because the slides were easier, and usually quicker, to read, and they had greater confidence in the assessments they reported. Obviously, specialized staining methods have their place in particular applications—for example, the assessment of acrosomal status.

It should also be noted that, although rapid methods such as Diff-Quik might permit reasonable preparations for a rapid assessment of normal forms (Menkveld et al, 1997b), they cannot provide all the insights that can be obtained from Papanicolaou-stained smears—for example, the differentiation of midpiece defects and cytoplasmic droplets. Especially when we consider the great clinical significance now attached to sperm morphology results, professional pride urges that the best available technique be used.

Finally, it must be stated categorically that reliable clinical assessments of human sperm morphology *cannot* be performed on unstained preparations, even at high magnification or by use of special optics such as Nomarski differential interference contrast, and any attempt to assess morphology on motile spermatozoa in wet preparations (eg, in a Makler chamber) is completely ineffective for identifying many important defects and must be considered totally unreliable.

Current Classification Schemes for Sperm Morphology

Of the sperm morphology classification schemes listed in Table 1, the David system (1975) is hardly used outside France, and the Dusseldorf system (Hofmann et al, 1985) is restricted almost exclusively to some German centers.

Table 1. *Summary of major recent sperm morphology classification systems*

Classification System	Source References
MacLeod	MacLeod, 1964, 1970
David (France)	David et al, 1975
WHO'80 (1st edition)	Eliasson, 1971; Belsey et al, 1980
Dusseldorf	Hofmann et al, 1985
Tygerberg Strict Criteria	Kruger et al, 1986; Menkveld, 1987; Menkveld et al, 1990
WHO'87 (2nd edition)	World Health Organization, 1987
WHO'92 (3rd edition)	World Health Organization, 1992
WHO'99 (4th edition)	World Health Organization, 1999

Consequently, the three principal schemes currently in use internationally are the Tygerberg Strict Criteria and those described in the 1992 and 1999 versions of the WHO laboratory manual (World Health Organization, 1992, 1999). Because the WHO schemes represent evolutions of the earlier editions of the WHO laboratory manual (Belsey et al, 1980; World Health Organization, 1987), which in turn were based largely on the work of MacLeod (1964, 1970) and Eliasson (1971), they will be considered first, even though the work on which the Tygerberg Strict Criteria were based was already under way at the end of 1970s. Interested readers seeking more extensive historical background are referred to reviews and books by Eliasson (1971, 1981), Mortimer (1994), Ombelet (Ombelet et al, 1995; Ombelet, 1998), and Coetzee (Coetzee et al, 1998).

WHO'92

As was already mentioned, the 1980 sperm morphology assessment guidelines published by the WHO were based on those proposed by MacLeod (1970), with some modifications. Greater emphasis was placed on immature germinal cells, and the categories of midpiece defect, cytoplasmic droplet, and tail defect were added to the original complement of small, megalo, pyriform, tapering, and acutely tapering (spindle-shaped, so-called stress cells) heads and bicephalic forms. In many regards, these descriptions had remained unchanged since the 1920s, '30s and '40s (Hammen, 1944). The WHO'80 manual (Belsey et al, 1980) also indicated that it had added pyriform to MacLeod's list, but this has already been shown in his earlier illustrations. Eliasson's classification system (1971) was the first to specify the use of morphometric values for sperm head length and width, but their origin was not explained. His intention was to have as few alternative classifications as possible, so as to make standardization possible and facilitate the instruction of others were excellent principles, but unfortunately his decision that ''borderline forms have to be counted as normal'' left

the door wide open for differences in interpretation of the scheme and in its application by different laboratories.

Clearly, these changes improved MacLeod's early classification, which was strongly biased toward the sperm head. However, in WHO'80, there was no clear textual explanation of the scoring system, only legends to the color plates (which remain some of the best printed color illustrations of human sperm morphology outside those in the atlas by Menkveld et al, 1991b), and there was no explanation on how to enter values into the sample semen analysis record form. Finally, the focus on immature germ cells in WHO'80 had little impact on the profession: very few workers were able to make the distinctions reliably and with confidence, and there was no indication of what the values meant or how they were to be applied clinically.

The second edition of the WHO manual (World Health Organization, 1987) did little to clarify the assessment of sperm morphology. Indeed, its inclusion of a ''simplified Papanicolaou'' staining method and the statement that sperm morphology ''can be examined conveniently under phase-contrast microscopy with a 40× objective'' was a severe backward step (cf Eliasson, 1971). Although the description of a normal spermatozoon was improved, it was still only included in a plate legend, and, again, there was no instruction on how to record the results in the sample form. Consequently, no real improvement in standardization of sperm morphology assessments was achieved by WHO'87.

Consequently, one of the major goals on the third edition (World Health Organization, 1992) was to remove ambiguity from descriptions and try to eliminate possible sources of variation in application and interpretation of the various components of the semen analysis. The scheme for the morphological classification of human spermatozoa was described carefully, but the illustrations were essentially useless, being in monochrome and at far too low magnification. However, particularly in response to the study by Jouannet et al (1988) on the predictive value of an index of the incidence of multiple anomalies in abnormal spermatozoa, the decision was taken to adopt, and explain the application of, a multiparametric classification system. It was clearly stated that abnormal spermatozoa could have more than one defect and that the extent of this occurrence was prognostically useful. It seems likely that multiparametric scoring had been intended in earlier editions of the WHO manual (see Eliasson, 1981), but its implementation was never clarified, and Eliasson's role in this evolution of human sperm morphology scoring has been largely unknown except to those who have made sperm morphology assessment a specific career interest.

The decision to restrict the WHO'92 defect categories to four (head, neck/midpiece, tail, and cytoplasmic droplet) was a practical one. As Eliasson commented in 1971, restricting the number of possible classifications facilitates better standardization. Put simply, the committee (of which DM was a member) agreed that it was not considered important to know whether a sperm head was too small or too large, or whether it was tapering or pyriform (or both, in which case which group should it be scored in?), but that the important observation was that the spermatozoon in question had a morphologically abnormal head. It was believed that a highly simplified scheme would be more readily accepted, since very few labs performed any type of multiparametric assessment and that the more complicated any proposed system was, the less likely it would be accepted. Also, such a system would be far easier to teach and implement. However, should any particular sperm abnormality be common, then it was to be noted on the report, eg, pinheads.

The descriptions in WHO'92 were prepared by one of the authors (DM) and reflected his work on human sperm selection in vivo and in vitro carried out during the second half of the 1970s. The intention was to define abnormal spermatozoa by the presence of one or more defects that, from experience, could be expected to impair a spermatozoon's functional ability, particularly with regard to the penetration of cervical mucus. In applying this system, normal spermatozoa were identified by default, in that they had no recognizable defect that might be expected to affect their function. Hence, although it was stated that borderline forms should be considered as abnormal, since it is always easier to standardize strictness than leniency, abnormality was not based on some anthropomorphic concept of ''a nice looking sperm'' but rather on associations between sperm morphology and function.

It was discussed by the committee responsible for WHO'92 (meeting in Geneva in the summer of 1990) whether the Tygerberg scheme should be adopted by the WHO for sperm morphology. However, because the available data only related to IVF success, and because a shift from the ''normal value'' of 50% or more with normal morphology defined in WHO'87, it was felt that shifting to a cutoff of 14% was too big a move for the professional community. The value of 30% normal forms described in WHO'97 was accepted by consensus. However, there was a qualifier attached to this value, that ''[a]lthough no clinical studies have been completed, experience in a number of centres suggests that the percentage of normal forms should be adjusted downwards when more strict criteria are applied. An empirical reference value is suggested to be 30% or more with normal forms.'' Some clinical studies were planned but were severely affected by budget cuts that affected the WHO's Human Reproduction Programme soon afterward. Although some studies have been published purporting to compare WHO'92 with oth-

er schemes (eg, Morgentaler et al, 1995), there is no evidence that specific training in the WHO'92 scheme was applied before embarking on them, and so they will not be discussed here.

Hence, the ''multiple anomalies index'' of Jouannet et al (1988) became the ''teratozoospermia index'' (or TZI). But it was not a ''nonsense index'' like the total normal motile sperm count (obtained by multiplying the sperm count by the percentages of motile and normal spermatozoa, which themselves are not independent subpopulations) but rather is a simplification of the expression of the negative aspect of sperm morphology to facilitate its interpretation by clinicians. In this regard, it was similar to MacLeod's ''motility index,'' in which percentages of sperm showing different degrees of motility are multiplied by a grading factor to create a single value. Such indices are extremely valuable to many clinicians when they try to make sense of all the numbers included in a semen analysis report; this is not something that the laboratory necessarily wants (or even uses in many instances) but something that the clinician requesting the test needs. Adverse opinions on the use of such indices should be directed to clinicians, not laboratory scientists, and used to identify educational needs in the training of clinical andrologists. Baying at the moon and tilting at windmills do not help science advance, especially in clinical science, in which the laboratories are constrained to provide reports that the referring clinicians feel able to understand and interpret.

The TZI was, therefore, the ''average number of defects per abnormal spermatozoon'' and hence an indirect indication of 1) the risk of what appeared to be normal spermatozoa actually having defects that were invisible at the level of observation and 2) just how badly affected spermiogenesis was in the man, and hence how impaired his sperm fertilizing ability might be. Just as the percentage of ''normal'' forms only reflects normality in terms of the level of the observation being made, the TZI represents another, negative, dimension of the assessment of sperm morphology at the light microscope level. Of importance is the fact that it provides an extra dimension of information to help understand the likely significance of situations in which there are very few normal forms. TZI introduces a dynamic range into our interpretations when the difference between 4% and 6% normal forms is considered to reflect a major difference in clinical significance. This is especially important when one remembers that even those labs that count 200 spermatozoa cannot achieve better discrimination than ±7%—ie, values of 3% and 9% normal forms are not statistically different.

It is in this aspect of clinical management that the TZI is of greatest value, and, although it has been reclassified as an ''Optional Test'' in WHO'99 (see below), it is integral to the management decisions considered in the revised edition of the WHO's clinical manual, *WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male* (Rowe et al, 2000).

WHO'99

When updating the laboratory manual to its fourth edition (World Health Organization, 1999), the WHO decided to adopt the Tygerberg Strict Criteria for sperm normality but retained the functional defect-based approach to scoring, making for a rather confused situation. The recommendations of careful Papanicolaou staining and examination by use of a 100× bright field objective were retained, and the number of spermatozoa to be counted was changed from *''at least 100, and preferably 200''* (WHO'92) to *''at least 200 consecutive spermatozoa are counted''*—with the additional comment that *''when the diagnosis and treatment of the patient crucially depends on the percentage of spermatozoa with normal morphology, 200 spermatozoa should be assessed twice to increase the precision.''*

The TZI was reclassified as an ''Optional Test,'' and, strangely, cytoplasmic droplets were removed from its calculation, giving it a range of 1.00 to 3.00. This is particularly surprising, since on page 21 of the manual, cytoplasmic residues are defined as an abnormality. Given our knowledge of the significance of residual cytoplasm in the detrimental ability of spermatozoa to generate reactive oxygen species, such an arbitrary change is difficult to understand.

Better color plates were included, but no reference value for sperm morphology was given. Instead, the following statement was made: *''[m]ulticentre population-based studies utilizing the methods of morphology assessment in this manual are now in progress. Data from assisted reproductive technology programmes suggest that, as sperm morphology falls below 15% normal forms using the methods and definitions described in this manual, the fertilization rate in vitro decreases.''*

Consequently, WHO'99 can be seen as an integration of the WHO'92 and Tygerberg Strict Criteria classification systems. A major improvement has been the shift toward a lower cutoff for the percentage of normal forms and especially in the redefinition of this number as a ''reference value'' rather than a ''normal value.'' But, in reality, has anything actually changed? The next section will deal with the development and evolution of the Tygerberg Strict Criteria and is followed by a blind comparative study between the WHO'92 and Tygerberg systems that was carried out in the mid-1990s.

The Tygerberg Strict Criteria

Historical Background

The evaluation of the postcoital test (PCT) has been a standard procedure in the investigation of the infertile couple at the Andrology Clinic, Tygerberg Hospital, since its inception in about 1970. For the purpose of the PCT, samples were obtained 2 hours after intercourse from the vaginal pool, the ectocervix, and the endocervix (Van Zyl, 1975, 1980). These samples were sent to the laboratory on separate microscope slides for investigation of the number of spermatozoa per high-power (40×) field, motility, and speed of forward progression. With his interest in sperm morphology, RM, as early as the mid-1970s, also made smears from some of the samples for morphology evaluation of the spermatozoa present in the different fractions. Smears were stained by the Papanicolaou staining method and evaluated for normal sperm morphology under 100× oil immersion. When the results of the three different fractions were compared, it was obvious that there was a marked improvement in the percentage of morphologically normal spermatozoa. A mean of more than 90% normal spermatozoa was observed in excellent endocervical mucus fractions, whereas the morphology of spermatozoa in the seminal pool fractions resembled that of the original semen samples (Menkveld and Stander, unpublished data). These findings strengthened RM's beliefs that the criteria for sperm morphology evaluation were too lenient and that new criteria were needed for what should be considered as morphological normal spermatozoa.

Before RM visited the laboratory of Prof. David, where he met DM, as mentioned above, he first visited Prof. Rune Eliasson's laboratory in Stockholm, Sweden, with one of his main objectives being the study of sperm morphology. From the outset, it was clear that stricter criteria were already being applied by RM because, in his initial comparison of 6 semen smears from Eliasson's laboratory, RM's evaluations were significantly stricter than those determined by Eliasson, with mean values of 45.2% and 57.3% morphologically normal spermatozoa, respectively (unpublished data).

After his return from his 3-month visit to 6 European laboratories, RM started work on his PhD dissertation (Menkveld, 1987), in which the repeatability of sperm morphology evaluation was an important aspect. Emphasis was placed on the importance of well-defined criteria for normality on the basis of the appearance of spermatozoa as found in mucus from the endocervical channel, as well as the necessity for preparing good smears, good staining, and standardized evaluation methodology. In the meantime, the article by Kruger et al (1986) was published, illustrating the clinical importance of the stricter application of sperm morphology evaluation criteria based on the principles of RM's dissertation.

The chapter from RM's PhD dissertation on sperm morphology evaluation by stricter criteria (Menkveld, 1987) was first submitted on 16 January 1987. The manuscript was rejected with a note from the editor, dated March 30, 1987, stating *''[m]y inclination is to say that we cannot publish the paper since it contributes little if anything that is new to the field; however, if you feel that you can add new data to the manuscript and/or substantially modify it to give a more pertinent message then I would be prepared to reconsider it.''* The manuscript was resubmitted on June 5, 1989, but by then that particular journal had ceased publication. It was then submitted to *Human Reproduction,* and, after some modifications, accepted for publication on March 22, 1990 (Menkveld et al, 1990).

Rationale

As mentioned previously, criteria for what was thought to be a ''normal'' human spermatozoon were derived by consensus of several opinions (Freund, 1966) or based on the description of the modal sperm form seen in ejaculated semen samples of so-called fertile groups and individual men. However, humans are one of a small group of species whose semen specimens exhibit extreme heterogeneity or pleiomorphism of sperm morphology, both between (Menkveld et al, 1990, 1991b; Mortimer, 1994), and even within, individuals (Hartmann et al, 1964; MacLeod, 1970). Consequently, application of this approach for defining normal spermatozoa has been unsuccessful in the human, leading to large differences being found between and even within laboratories when sperm morphology is evaluated according to these criteria (Freund, 1966; Jequier and Ukombe, 1983).

Another approach was to define different classes of abnormal spermatozoa and then consider those forms that could not be classified into one of the abnormal groups to be the normal sperm population, the so-called liberal approach (Comhaire et al, 1994). However, this approach also has its shortcomings, since it will lead to the presence of both a population of true morphologically normal spermatozoa and a population that is thought to be normal but, according to biological evidence, should have been considered as abnormal (Menkveld and Kruger, 1995, 1996; also see below).

The introduction of the Tygerberg Strict Criteria brought a new concept into the field of sperm morphology evaluation, since, for the first time, the description of a so-called normal spermatozoon was based on biological criteria of spermatozoa selected by physiological principles governing their migration through endocervical mucus. This selective capacity has been known for many years; as early as 1930, Cary stated that the morphology

of human spermatozoa bears a definite relation to their success of migration through cervical mucus. This statement was repeated by Cary and Hotchkiss (1934) when they wrote *''[a]bnormal forms may possess motility but are rarely if ever found in the upper levels of cervical mucus and we must consider them ineffectual for fertilization.''* Furthermore, it was shown by Mortimer et al (1982) and Fredricsson and Björk (1977) that strong selection of certain morphological types of spermatozoa occur with migration through cervical mucus and that the morphological normality of these populations are significantly increased and, moreover, of strong prognostic significance (Fredricsson and Björk, 1977). However, the concept of using the appearance of these spermatozoa as a reference population to describe the ideal ''normal'' spermatozoon was not proposed at that time.

The Tygerberg Strict Criteria for an ideal normal spermatozoon are therefore based on the morphology of postcoital spermatozoa found at the level of the internal cervical os. In most cases, an apparently homogeneous sperm population is found here, in contrast to the heterogeneous sperm populations found in the first or lower part of the endocervical canal, as indicated by the presence of epithelium cells (Menkveld et al, 1990). The morphology of the spermatozoa at the internal os formed the basis of the Tygerberg Strict Criteria for the ideal morphological normal spermatozoon. However, even in such an apparently homogeneous population, a small range of normal biological sperm variants can be found. Excluding the obviously abnormal forms such as small and elongated spermatozoa, these forms must also be included in the range of normality. However, to ensure that the abovementioned normal variants are kept as small as possible, and thereby ensure repeatable evaluations, Tygerberg Strict Criteria (1990), in contrast to other evaluation systems (Freund, 1966; Eliasson, 1971; Belsey et al, 1980; Mortimer, 1985; World Health Organization, 1987), regard spermatozoa with so-called borderline or slightly abnormal head forms as abnormal.

Description

The definition for a morphologically normal spermatozoon by strict criteria is as follows. The head must have a smooth oval configuration with a well-defined acrosome constituting 40%–70% of the anterior sperm head. A second, slightly differing type of normal head form is also recognized: an oval form still having a smooth regular contour but which is slightly tapered (but not elongated) at the posterior end, as has been described elsewhere (Williams, 1937; Amelar et al, 1973). Sperm dimensions are based on those suggested by Eliasson (1971): the normal head length is between 4 and 5 μm and width between 2.5 and 3.5 μm. The head width must be between three-fifths and two-thirds the head length. No neck, midpiece, and/or tail defects may be present. The midpiece must be slender, axially attached, less than 1 μm in width, and approximately 1.5 times the normal head length. Cytoplasmic droplets (remnants) that constitute more than 30% the size of a normal sperm head are regarded as abnormal. Tails must be straight, uniform, slightly thinner than the midpiece, uncoiled, and about 45 μm long. So-called borderline normal spermatozoa are classified as abnormal. To be considered as normal, the whole spermatozoon must be normal, as was also stated by Eliasson (1971).

The guidelines of the Tygerberg Strict Criteria (Menkveld et al, 1990) for a morphologically normal spermatozoon are further supported by the morphological appearance of spermatozoa found tightly bound to the human zona pellucida, as seen in the hemizona assay (Menkveld et al, 1991a) and other in vitro sperm-zona binding tests (Liu and Baker, 1992). The morphology of the tightly bound spermatozoa closely resembles those of spermatozoa found in good postcoital cervical mucus and is a further indication for the natural selection of spermatozoa defined as morphologically normal by the Tygerberg Strict Criteria during gamete approximation and interaction.

The Tygerberg Strict Criteria method was originally developed as a laboratory technique based on the in vivo situation (Van Zyl et al, 1976, 1990; Menkveld, 1987). It has since been applied to in vitro studies (Kruger et al, 1986) and is thus applicable to both the in vivo and in vitro situations. Morphology evaluation by strict (Tygerberg) criteria is a holistic approach. The ''strict'' does not only refer to a single aspect such as the criteria for sperm normality; the method is also stricter with regard to the range of normal variations allowed and stricter in that slightly abnormal or borderline normal forms must be regarded as abnormal. The method is also stricter with regard to the preparation of the semen smears, the staining of the smears, and the methodology used for the evaluation process (Menkveld et al, 1990; Menkveld and Kruger, 1996).

The Acrosome Index

With the strict (Tygerberg) criteria, the appearance of the acrosome plays an important role in defining the ideal normal spermatozoon. Although slightly abnormal and elongated spermatozoa may have normal acrosomes, these cells are recorded as abnormal according to strict criteria. These types of spermatozoa have, as mentioned above, been observed at the endocervical os and also bound to the zona pellucida. In some cases with severe teratozoospermia or a P-pattern (<4% morphologically normal spermatozoa), good fertilization rates can still occur (Kruger et al, 1988). With further basic investigations, Menkveld et al (1991b) found that two distinct acrosomal

morphological patterns could be observed between the groups in which fertilization did and did not occur. In the few cases with good fertilization, it was striking to observe a pattern of slightly and moderately elongated spermatozoa but with morphologically normal (size and form) acrosomes. In the group with no or very poor fertilization in vitro, it was observed that, in the majority of the cases, small and/or abnormal acrosomes were the dominant abnormalities. In a further study (Menkveld et al, 1996), it was found that when the acrosome morphology was classified into four different groups—ie, normal, small, staining defects, and amorphous—and the results expressed as the acrosome index (AI = % of normal acrosomes), no fertilization occurred when the AI was less than 15%. In a follow-up study, it was found that the AI cutoff value could be set at 8% normal acrosomes (Menkveld et al, 1998). Because these two studies showed that the particular type of acrosomal abnormality did not play a role in fertilization outcome, only the presence of morphologically normal acrosomes is now recorded.

Sperm Acrosomal Morphology Evaluation

For the evaluation of the acrosome morphology, the same principles are applied as those for the evaluation of normal sperm morphology according to strict criteria. For an acrosome to be regarded as normal, the acrosome must have a smooth normal oval shape, with the same dimensions as for a normal spermatozoon. Acrosomes must be well-defined and constitute about 40%–70% of the normal-sized sperm head. The postacrosomal part of the sperm head can be abnormal, but the rest of the spermatozoon must be normal—thus, no neck, midpiece, and tail abnormalities and no cytoplasmic residues may be present. If the spermatozoon is classified as normal, the acrosome must always be classified as normal. The acrosome evaluation can be performed simultaneously with the routine morphology evaluation, with the aid of two laboratory counters. On the first counter, the sperm morphology is scored as normal or abnormal. The first key of the second counter is pressed if an acrosome is considered to be normal. As with the normal sperm morphology, at least 100 spermatozoa are evaluated. The AI will always be larger than the percentage of morphologically normal spermatozoa. If only one laboratory counter is available, the AI can be determined after the normal morphology evaluation, by counting another population of spermatozoa and scoring their acrosomes as normal or abnormal and expressing the result as the percentage of normal acrosomes, ie, the AI.

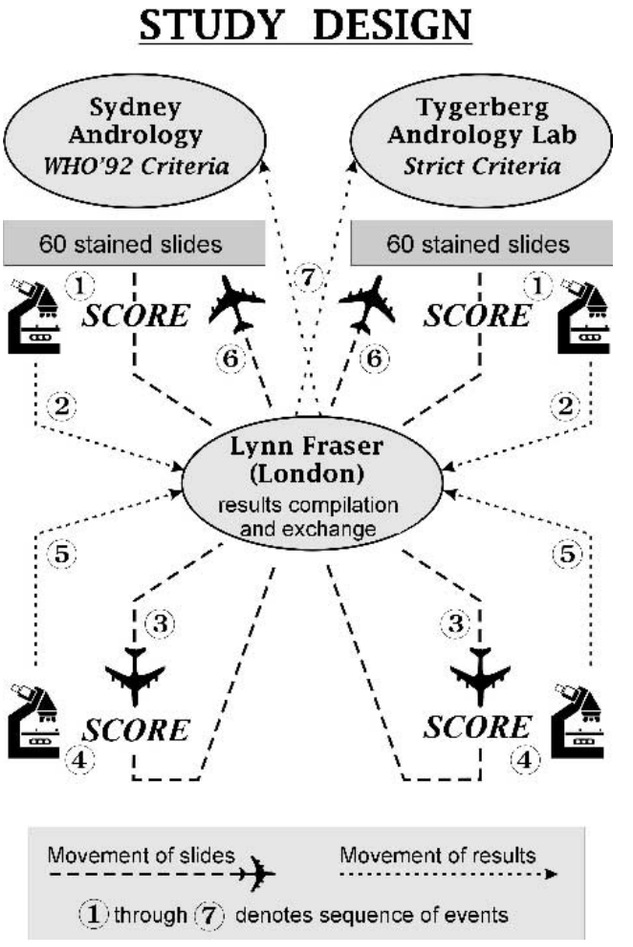

Figure 2. Diagrammatic representation of the organization of the authors' crossover study comparing WHO'92 criteria and Tygerberg Strict Criteria for human sperm morphology assessment.

A Comparison of WHO'92 and Tygerberg Sperm Morphology Criteria

This study was undertaken by the authors' laboratories in Sydney and Tygerberg and reported at the 1997 congress of the South African Society for Reproductive Science and Surgery (Mortimer et al, 1997). Sydney Andrology and the Tygerberg Andrology Laboratory each provided 60 Papanicolaou-stained and mounted slides of seminal spermatozoa for assessment by both laboratories (see Figure 2). After scoring in their originating laboratory, the sets of slides were exchanged and scored at the other site. Results were exchanged through an independent third party (Prof. Lynn Fraser, London), but only after all results were in the third party's possession; neither laboratory was able to reassess the other's slides after reporting their results. Each laboratory used their standard classification criteria for human sperm morphology according to routine clinical practices. It was judged that since DM had been

Table 2. *Results of the authors' cross-over study comparing WHO'92 criteria and Tygerberg Strict Criteria for human sperm morphology assessment. Values are mean ± SD*

	Strict Criteria	WHO 1992 Criteria					
Source of Material	Normal forms	Normal Forms	TZI*	Head Defects	Neck/midpiece Defects	Tail Defects	Cytoplasmic Droplets
Sydney	10.9 ± 6.6	6.4 ± 4.4	1.64 ± 0.17	89.7 ± 5.7	49.8 ± 11.2	12.8 ± 8.0	1.4 ± 1.0
Tygerberg	9.5 ± 4.5	3.1 ± 2.2	1.55 ± 0.18	96.2 ± 2.7	44.0 ± 13.1	10.7 ± 7.2	0.1 ± 0.1
Overall	10.2 ± 5.7	4.7 ± 3.8	1.59 ± 0.18	93.0 ± 5.5	46.9 ± 12.5	11.7 ± 7.7	0.7 ± 1.0

* TZI denotes teratozoospermia index.

responsible for preparing the description of how sperm morphology should be assessed for the third edition of the WHO manual (ie, WHO'92), Sydney Andrology could be taken as a valid, if not definitive, application of this scheme, and because RM was responsible for the development of the Strict Criteria at Tygerberg, his assessments could also be taken as definitive.

Overall, the range of semen specimens represented in the two sets of slides were not different (Table 2), but, unexpectedly, the careful application of the WHO'92 criteria by Sydney Andrology was actually found to be more critical in assigning morphologic normality than the Tygerberg laboratory's application of their Strict Criteria ($P < .001$). Because the WHO scheme incorporated a detailed assessment of head, neck/midpiece, tail, and cytoplasmic droplet regions for defects, the statistical analysis was repeated comparing WHO percentage normal sperm heads with Tygerberg percentage normal forms, which showed a slightly reduced, but still highly significant, difference between the two morphology classification schemes (Table 3, $P < .001$).

From these results, it was clear that if the WHO'92 classification scheme was applied strictly, then it was little different to the Tygerberg scheme. This is especially important in the interpretation of the proportion of normal forms, in which the 1992 recommended WHO cutoff of 30% was clearly too high if sperm normality is assessed critically. On the basis of the results, the authors concluded that although further data would be required to establish whether there may be a greater difference between the two schemes when considering normal fertile men and where ''borderline'' forms might be more prevalent, the development of a unified approach for assessing human sperm morphology should not be insurmountable. A final, cautionary, note from this study is that all people assessing human sperm morphology must be aware of the risk of becoming too strict.

Table 3. *Results of the authors' cross-over study comparing WHO'92 criteria and Tygerberg Strict Criteria for human sperm morphology assessment but comparing Tygerberg normal forms against WHO (100 − % head defects) only. See text for further explanation. Values are mean ± SD*

Source of Material	Tygerberg % Normal Forms	Sydney (WHO'92) 100 − % Head Defects
Sydney	10.9 ± 6.6	10.3 ± 5.7
Tygerberg	9.5 ± 4.5	3.8 ± 2.7
Overall	10.2 ± 5.7	7.0 ± 5.5

Clinical Significance of Sperm Morphology Assessments

The clinical value of the Tygerberg Strict Criteria as a prognostic tool in IVF was originally demonstrated in the publication by Kruger et al (1986) on 190 treatment cycles. With more than 15% morphological normal spermatozoa, the fertilization rate (of metaphase II oocytes only) was 82.5%, with a pregnancy rate of 25.8% (>3 embryos transferred). When the percentage of normal spermatozoa was 14% (22 cases), there was a drastic fall in the fertilization rate to only 37%, and no pregnancies were achieved. In 1988, Kruger et al published a second paper in which they reported the results of a study specifically investigating a group of men with less than 15% morphological normal spermatozoa. They found that two subgroups could be distinguished; those with a good prognosis (G-pattern), having between 14% and 5% normal spermatozoa, and those with a poor prognosis (P-pattern) having 4% or less normal spermatozoa, with fertilization rates of 63.9% and 7.4%, respectively. Furthermore, for the in vivo fertilization situation the same lower cutoff value of 4% or less morphological normal spermatozoa has been established (Van Zyl et al, 1990).

The positive predictive value of strict criteria has subsequently been confirmed by many other publications (Oehninger et al, 1988; Enginsu et al, 1991, 1992, 1993; Grow et al, 1994; Ombelet et al, 1994; Vawda et al, 1996), although there have been occasional publications with negative reports (Morgentaler et al, 1995).

To compare the clinical value of strict criteria with other morphology evaluation methods in assisted reproduction with regard to fertilization and pregnancy rates, Coetzee et al (1998) conducted a literature search and performed a meta-analysis on the accumulated data. Articles from between 1980 and 1996 dealing with sperm morphology evaluation according to any criteria or methods

were collected if they provided morphology thresholds, true oocyte fertilization rates, ongoing pregnancy rates per cycle, and pregnancy rates per transfer. From 216 articles examined, 54 were included, with 44 reporting a positive predictive role for sperm morphology and 9 a negative role. Results of only 22 articles could be used for statistical analysis, 19 on the basis of strict criteria and 3 on WHO criteria. All 3 articles that used WHO criteria reported a positive role for sperm morphology, with 2 showing significant odds ratios (ORs) and 2 a predictive value for pregnancy outcome. As far as strict criteria were concerned, all 5 articles that used the 15% or greater threshold reported a positive role for strict criteria, all with significant ORs, and 6 of 8 articles reported a significant role with regard to pregnancy outcome, with 2 showing significant ORs. All 10 strict-criteria articles using a 5% threshold reported a positive role for prediction of fertilization outcome (9 with significant ORs), and 8 of 11 showed a positive predictive role for pregnancy outcome (3 with significant ORs). Coetzee et al (1998) reported that the combined fertilization rate for all strict criteria articles was 59.3% and 77.6% in the 4% or less and 5% or greater normal sperm morphology groups, respectively. The combined pregnancy rates for strict criteria were 15.2% and 26.0% in the 4% or less and 5% or greater groups, respectively. Coetzee et al (1998) also reported that when the percentage of morphologically normal spermatozoa according to strict criteria was 4% or less of the combined proportion of cycles where no embryo transfers were performed was 25.5%, compared with only 7.5% in the 5% or greater normal sperm morphology group. To summarize, Coetzee et al (1998) found that, irrespective of the morphology evaluation method used, 44 of the 54 articles concluded that morphology evaluation could play a positive predictive role with regard to fertilization outcome in IVF and, to a lesser degree, pregnancy outcome. However, strict criteria at both the 5% and 14% thresholds had a stronger predictive value, compared with WHO criteria, where no consensus has yet been reached on a threshold value.

The lower predictive value obtained with WHO (Belsey et al, 1980; World Health Organization, 1987, 1992) criteria compared with strict criteria may, to some extent, be due to the fact that when sperm morphology is assessed according to the liberal approach (World Health Organization, 1987; Comhaire et al, 1994) the ''normal'' sperm population will actually be composed of two populations of spermatozoa. One will be the truly normal population and the other an abnormal group that had not been identified as such. Therefore, poorer correlation should be expected between the percentage of morphologically normal spermatozoa and in vitro fertilization rates or functional tests, compared with strict criteria. These theoretical disadvantages of the liberal approach are supported by reports in the literature stating that abnormal sperm morphology was found to be less sensitive for the evaluation of an ejaculate, compared with other semen parameters (Van Duijn et al, 1972). Page and Holding (1951) found no correlation between abnormal morphology and pregnancy outcome, and Hellinga (1976) found that abnormal morphology was of less importance in comparison with normal morphology in the prediction of male fertility potential.

The difference in the predictive value between strict criteria and WHO (1987) criteria for in vitro fertilization outcome was illustrated by the work of Oehninger et al (1988). When the sperm morphology of a group of patients with no fertilization after IVF was evaluated according to the WHO criteria, only 32.7% of cases could be attributed to poor morphology, and 40.4% of cases were attributed to an unexplained factor. However, when sperm morphology was reevaluated according to strict criteria, the sperm morphology factor group increased to 61.5%, and the unexplained group was reduced to only 11.5%.

According to Coetzee et al (1998), a possible overriding factor favoring strict criteria may rather be the level of commitment to using sperm morphology evaluation in male infertility diagnosis. This commitment is reflected in the implementation of good inter- and intraobserver and -laboratory quality control, and the establishment and use of clinically based normal sperm morphology descriptive guidelines and fertility thresholds. Adherence to these basic principles has helped to establish the Tygerberg Strict Criteria as a dependable diagnostic tool. Coetzee et al (1998) further stressed that although the clinically based thresholds for strict criteria have been refined to include the poor- and good-prognosis groups (Kruger et al, 1988) and, more recently, the AI (Menkveld et al, 1996), the physiological basis for strict criteria (Menkveld, 1987; Menkveld et al, 1990) has remained constant, in contrast to the WHO standards, which have changed constantly, in an apparently arbitrary manner.

It is important to reemphasize, as mentioned earlier, that in no study was normal sperm morphology found to be the only predictive semen parameter with regard to fertilization and pregnancy outcome. Considering the complex sequences leading to these events, this should be entirely expected.

Finally, with the acceptance by the WHO of the Tygerberg Strict Criteria for sperm morphological normality (World Health Organization, 1999), there is now a single reference method for human sperm morphology assessment. The value and widespread adoption of additional facets of sperm morphology such as the AI and/or teratozoospermia index will remain to be seen. Their particular value might lie in refining the decision points used to optimize patient management, especially in cases

where the proportion of normal spermatozoa lies between 5% and 14% (ie, the G-pattern group) when using the concepts described in the *WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male* (Rowe et al, 2000). However, it cannot be stressed enough that reliable implementation of Tygerberg Strict Criteria cannot be achieved solely from reading a book. Practical training courses, both external and in-house, are essential, and must be combined with continual, rigorous internal quality control and participation in effective external quality assurance programs. Only by these means can interlaboratory standardization and consistency be achieved and the pitfall of progressive excessive strictness be avoided.

Conclusions

We hope that this explanation of the historical origins of human sperm morphology assessment and the classification schemes currently used in the majority of centers around the world will dispel some of the myths surrounding the clinical significance and application of these results. A better understanding of just what is being assessed by a sperm morphology count will eliminate confusion about the relationship between normal forms and fertilizing ability. Basing our assessments on sperm functional criteria rather than anthropomorphic perceptions of ''nice looking sperm'' now permit biologically useful opinions to be reached. Finally, how such results can be incorporated into clinical management protocols as decision points—eg, when ICSI will be required rather than IVF, or when IUI has a good expectation of pregnancy such that IVF is least cost-effective, will help andrology laboratories justify the need to perform careful assessments using the best available techniques. As professionals, we must always strive to provide the highest standards of patient care and not submit to constraints requiring the use of poorer techniques based on quasieconomic arguments intended only to cut budgets or increase profitability. The desire for best practice must be paramount.

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