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REVIEW ARTICLE

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Sperm morphology: assessment, pathophysiology, clinical relevance, and state of the art in 2017

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SUMMARY

For over 30 years, sperm morphology assessment has been one of the most common tests in evaluation of fertility. This review examines the clinical relevance of sperm morphology assessment in the diagnosis of infertility and in assisted reproductive technology, as well as its analytical reliability. Publications on the pathophysiology, the analytical reliability of the test and its clinical relevance in diagnosis and in Assisted Reproductive Technology (ART) were evaluated. This review compared and discussed study methodologies and results, including patient characteristics, preparation, smear staining methods and classification systems. The assessment of the percentage of some abnormalities such as for example thin head, amorphous head, or bent or asymmetrical neck is of little clinical use, and their pathophysiology is not well explained as most are physiological traits. Some studies have highlighted correlations between the percentage of normal forms and functional sperm abnormalities, as well as correlations with ability to conceive in vivo and, in some situations, with the success of intra-uterine insemination (IUI) or conventional IVF. However, except in the case of some specific sperm defects (easy to detect with 99 or 100% of spermatozoa affected) and which are often linked to genetic disorders (globozoospermia, macrocephaly, decapitated sperm syndrome and fibrous sheath dysplasia), sperm morphology assessment has very poor sensitivity and specificity in the diagnosis of infertility. Moreover, there is very little evidence that indices of multiple sperm defects [sperm deformity index (SDI), teratozoospermia index (TZI), and multiple abnormalities index (MAI)] are relevant. Above all, many publications report a major lack of analytical reliability of this test, mainly in assessment of the details of sperm abnormalities. Many questions arise concerning how and when sperm morphology should be assessed, and how to interpret the thresholds of normal forms. Questions are raised on the real clinical impact of this test.

INTRODUCTION

In men, the transformation of spermatids during spermiogenesis is a key post-meiotic event contributing to major morphological reorganizations. Spermiogenesis concerns the reorganization of the nucleus, the development and positioning of the acrosome from the Golgi apparatus, the assembly of the tail structures and reorganization of the cytoplasm, and the terminal phase ends in the release of spermatozoa in the lumen of the seminiferous tubule. Morphology assessment under optical microscopy shows that morphological modifications during spermiogenesis are not very homogeneous in humans, generating spermatozoa with various morphologies. Therefore, the main question is: what is a normal spermatozoon? Observations of spermatozoa that have migrated through the mucus of the upper endocervical canal have helped to define a normal-shaped spermatozoon (Menkveld *et al.*, 1990). According to the strict criteria, the percentage of 'ideal spermatozoa' in men is very low. Assessment of sperm morphology is the most discriminating sperm parameter between two populations of fertile and infertile men (Ombelet *et al.*, 1997a; Guzick *et al.*, 2001) with, for the latter, a cut-off of 10% according to ROC curves and 5% by using the 10th percentile of the fertile population for the percentage of normal shapes.

For 20 years, sperm morphology assessment has been described by some authors as a good indicator of male fertility (Bonde *et al.*, 1998; Slama *et al.*, 2002) and in some situations of the success of intra-uterine insemination (IUI) or conventional IVF (Kruger *et al.*, 1988; Coetzee *et al.*, 1998; Gunalp *et al.*, 2001;

Van Waart *et al.*, 2001; Spiessens *et al.*, 2003; Nikbakht & Saharkhiz, 2011). For other authors, the test is not a relevant prognostic factor for spontaneous pregnancy. In a model for predicting spontaneous conception leading to live birth within one year after intake and based on data from both partners, sperm morphology was not included (Hunault *et al.*, 2004). In the vast majority of countries, this laboratory test is one of the most common andrological investigations. However, numerous publications have highlighted its analytical weakness with wide intra- and inter-laboratory variations (Matson, 1995; Eustache & Auger, 2003; Franken, 2003; Menkveld, 2013). Since there are different classifications and lack of technical standardization, it does not meet current analytical requirements as a standard validated biological test. Many questions arise concerning the test's clinical relevance, how sperm morphology should be assessed, the specific situations in which the test should be performed, and how to interpret the thresholds of normal forms provided by the studies on this topic.

There is a lack of support from clinicians (andrologists and gynecologists) for this test, related in part to its weak analytical reliability. It is urgent to standardize the practices for technical performance of this test and good clinical use. This would contribute to greater acceptance by prescribing physicians.

In order to better understand the place of sperm morphology in the assessment of the fertilizing ability of men, we aimed, through this narrative literature review, to evaluate its clinical relevance in addition to its analytical reliability.

Firstly, we describe the different classification systems, their associated reference values and the technical challenges of the test, and discuss publications on the analytical reliability of sperm morphology assessment. Secondly, we review data concerning the current pathophysiological knowledge of the various abnormalities described during sperm morphology assessment in order to investigate their value as indicators of andrological disorders. Finally, we discuss data concerning the prognostic relevance of sperm morphology assessment before assisted reproductive technology (ART).

MATERIAL AND METHODS

We conducted a narrative review of the relevant literature. The PubMed database was used to retrieve works published between January 1980 and July 2016 using the following search terms: sperm morphology, strict morphology, strict criteria, and teratozoospermia. The publications' titles, abstracts and reference lists were reviewed and only relevant publications (i.e. those reporting on the pathophysiology of sperm defects, the analytical reliability of sperm morphology assessment and its clinical relevance in fertility diagnosis and in ART) were evaluated (Fig. 1). This review examined, compared and discussed study methodologies and results, including patient characteristics, preparation, the smear staining method, and the used classification system used. As this was a narrative review, no review protocol or registration number were required. No specific funding was received.

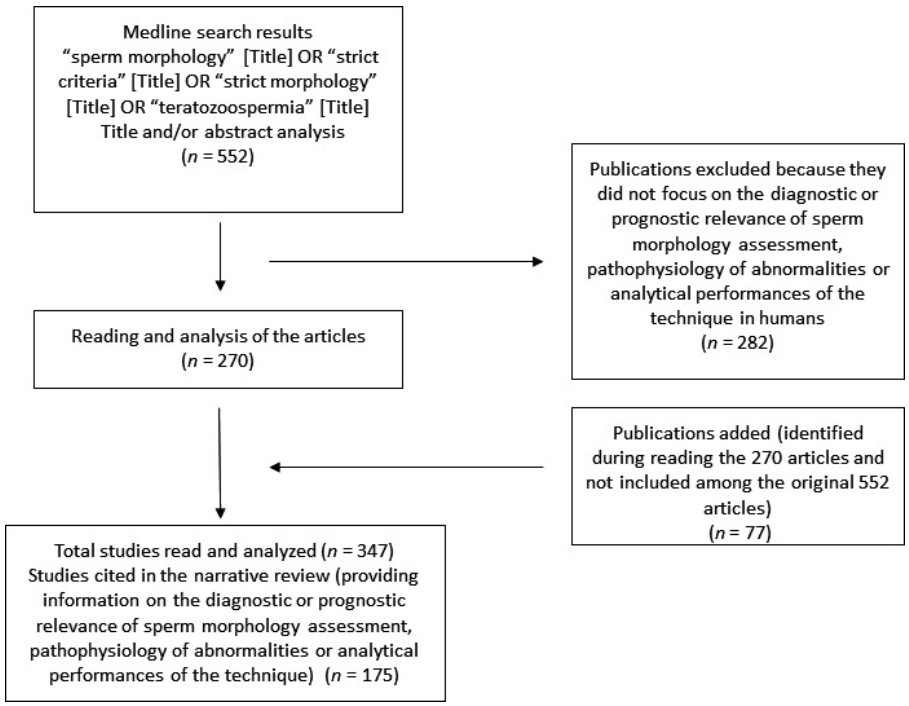
RESULTS

Classifications used to assess human sperm morphology

In 1982, spermatozoa recovered from cervical mucus and from the uterus and Fallopian tubes showed improved morphology compared with the spermatozoa in the original semen sample (Mortimer *et al.*, 1982). (Menkveld *et al.*, 1990) based their definition of a normal shaped spermatozoon on spermatozoa collected in cervical mucus.

Several classifications for sperm morphology assessment have been developed, and have different approaches. In the first approach described by MacLeod & Gold (1951), different

Figure 1 Flow chart for selection of relevant publications.



obvious abnormalities were described. All spermatozoa without clear, well-defined abnormalities were considered as normal: therefore, normal spermatozoa were identified by default. No specific criteria were given to define a normal spermatozoon. This liberal approach showed poor correlation with ability to conceive (Page & Houlding, 1951). It was used in the 1st and 2nd editions of the World Health Organization (WHO) manual (Table 1).

In contrast to this 'liberal' approach, strict criteria exist. Sperm morphology was described according to the Tygerberg strict criteria developed by Menkveld (Menkveld, 1987), and applied to in vitro study by Kruger *et al.* (1987b) based on observations made on spermatozoa present in the cervical mucus and described in detail by Menkveld *et al.* (1990). These descriptions were later supported by descriptions of spermatozoa bound to the human zona pellucida as seen in the hemizona assay (Menkveld *et al.*, 1991) and confirmed by in vitro sperm-zona binding tests (Liu & Baker, 1992). With the strict criteria, spermatozoa with slightly abnormal 'borderline' heads are classified as abnormal (Menkveld *et al.*, 1990). The range defining a normal form is small, and this is one of the most important aspects of this classification. The clear description of morphologically normal spermatozoa appears only in the 3rd edition of the WHO manual (Who, 1992) according to the Tygerberg strict criteria (Table 1). In the 4th edition of the WHO manual, the different abnormalities are enumerated but no precise description is given. The latest and 5th WHO manual (WHO, 2010) recommends using the strict criteria for identifying a normal spermatozoon and gives the following precise definition of a normal spermatozoon: '*The head should be smooth, regularly contoured and generally oval in shape… acrosomal region comprising 40–70% of the head area without large vacuoles, and not more than two small vacuoles… the post-acrosomal region should not contain any vacuoles…the midpiece should be slender, regular and about the same length as the sperm head… Residual cytoplasm is considered an anomaly only when in excess, i.e. when it exceeds one third of the sperm head size… principal piece with uniform calibre along its length, must be thinner than the midpiece, and approximately 45 μm long (about 10 times the headlength)…*'. In this 5th edition, schematic drawings of head defects, neck and midpiece defects, tail defects and excess residual cytoplasm are given. Other classifications exist, but are only used in certain countries. For example, in France the most commonly used classification is the modified David classification (Auger *et al.*, 2001). This classification was originally developed by David *et al.* in 1975 and modified by Jouannet *et al.* (1988) by adding some abnormalities and by establishing the multiple abnormalities index (MAI). Contrary to the strict criteria, the modified David classification considers spermatozoa with 'sub-normal' or 'borderline' forms as normal. 'Borderline' forms are known to be responsible for greater inter- and intra-observer variability (Menkveld, 2010). Changes in the reference values in the literature are sometimes related to the introduction or exclusion of a 'borderline' appearance of the spermatozoon in defining its normality.

Reference values for the percentage of normal forms

The WHO threshold for the percentage of normal forms has been reduced from the 1st edition in 1980 to the 4th, and especially to the 5th edition (Menkveld, 2010) (Table 1). In the 1st edition, average normal morphology was 80.5% (range 48–98% calculated on 602 ejaculates from 72 fertile men). In the 2nd edition, the value of the percentage of normal forms was 50%, then 30% in the 3rd edition with the note 'An empirical reference value is suggested to be 30% or more…'. In the 4th edition, the reference value was 14% with a note 'Multicentre studies are now in progress. Data from assisted reproductive technology programmes suggest that, as sperm morphology falls below 15%… the fertilization rate in vitro decreases'. In the recommendations of the 5th edition (WHO 2010), the lower reference limit is 4%, lower than the recommendation in the 4th edition, and the recommended criteria for spermatozoa morphology assessment are also different from the 4th edition. In the 5th edition, the reference value of 4% for the percentage of normal forms is based on the 5th percentile of combined data resulting from recent publications using known and standardized methodologies (Cooper *et al.*, 2010). The latter threshold is in agreement with the results of studies which compared populations of fertile and infertile men and established a discriminating threshold using ROC curves (Ombelet *et al.*, 1997a; Menkveld *et al.*, 2001, 2011; Haugen *et al.*, 2006). The reasons for the decline of reference values are mainly the introduction of strict criteria (and the classification of 'borderline spermatozoa' as abnormal), while other authors have suggested there is a real decrease of normal forms due to a negative impact of environmental factors (Carlsen *et al.*, 1992). The first hypothesis was approved by Ariagno *et al.* (Ariagno *et al.*, 2011): after reevaluation of semen over a period from 1973 to 1989, they could not demonstrate a true decline in sperm morphology despite a great

Table 1 Methods and cut-off values for sperm morphology in the different WHO manuals

WHO edition	Year	Criteria	Methods of assessment	Cut-off for normal forms (%)	Calculation of cut-off values
1st	1980	Liberal approach	No clear description of normal forms, 'classification based on MacLeod's description'	80.5	Mean of fertile population (range = 48–98%)
2nd	1987	Liberal approach	No important difference compared with the 1st edition	≥50	No precise data
3rd	1992	Strict criteria	Clear description of normal spermatozoa according to strict criteria with well-defined sperm head lengths and widths and qualitative descriptions	≥30	Arbitrary value
4th	1999	Strict criteria	List of various abnormalities without accurate description	14	No precise value given, 'multicenter studies refer to >14% for IVF'
5th	2010	Strict criteria	Precise definition of normal spermatozoa and of different abnormalities	4	Lower reference limit (lower fifth centile value), data from fertile men

difference in the percentage of normal forms of the same patients assessed after changing criteria.

In the French modified David classification, the reference value for the percentage of normal forms was at first 30% and was then reassessed by Auger *et al.* (2016) at 23% in a study that described the frequency of the various sperm abnormalities of the head, midpiece and tail principal piece in a large group of fertile men (male partners of pregnant females).

The use of one or the other classification has a major impact on the assessment of the percentage of normal forms. For instance, studies that examined the impact of morphology on ART results were conducted with the strict criteria. Therefore, the published threshold for decision-making cannot be applied to other classifications. Blanchard *et al.* (Blanchard *et al.*, 2011) have shown that the David classification was not discriminant for the rate of fertilization by conventional IVF compared with assessment using the strict criteria in the computer-assisted sperm analysis (CASA) system. According to a French survey, there is considerable heterogeneity not only in the different reference thresholds used, but also between laboratories that claim to use standardized values from the literature; 33.6% of them use inadequate reference values for their classification (Gatimel *et al.*, 2016). Concerning the definition of reference values, it has been suggested that each laboratory could define its own standards (Matson, 1995) in view of the wide range of variability related to the technique and the operator and because of the considerable heterogeneity in the methods used from one laboratory to another. Sperm morphology assessment is undertaken by very few laboratories, at least in France (8.5%), probably due to the difficulty of obtaining a reference population.

Indices of multiple sperm defects

Three different indices have been proposed and defined:

- The multiple abnormalities index (MAI), used in the French modified David classification, is the average number of abnormalities per abnormal spermatozoon.
- The teratozoospermia index (TZI) is similar to the MAI, but a maximum of four abnormalities per abnormal spermatozoon are counted: one each for the head, the midpiece, the principal tail piece, and the residual cytoplasm, regardless of the real number of abnormalities per abnormal spermatozoon.
- The sperm deformity index (SDI) is the number of abnormalities divided by the total number of spermatozoa (normal and abnormal).

Data from the literature on the clinical relevance of these indices is very scarce; in more than 30 years, there have only been one or two publications for each index, which seems largely insufficient for routine daily use in all laboratories. The MAI (Jouannet *et al.*, 1988; Slama *et al.*, 2002) and the TZI (Menkveld *et al.*, 2001) relate to in vivo fertility and the SDI relates to fertilization rate (but not pregnancy rate) in conventional IVF (Aziz *et al.*, 1996). According to van Zyl & Menkveld (2006), the TZI has a low predictive value for spontaneous fertility and ART outcomes. Considering data from the literature, we question the relevance of the systematic development of these indices.

Analytical performances of sperm morphology assessment

Several authors have regularly drawn attention to the wide intra- and inter-laboratory variability of this test. In 1977, Eliasson was one of the first to emphasize the necessity of conducting analytical performance of sperm morphology assessment (Eliasson *et al.*, 1977). Several factors are responsible for this technical variability: heterogeneity in the preparation and staining techniques for the smears, in the classification systems used, and technician competency for an assessment that is necessarily subjective. Therefore, the authors highlight the need to homogenize smear preparations, reading techniques and classifications, and stress the importance of conducting quality controls and training programs and maintaining operator competency (Davis & Gravance, 1993; Comhaire *et al.*, 1994; Matson, 1995; Ombelet *et al.*, 1997b, 1998; Bonde *et al.*, 1998; Keel *et al.*, 2000; Eustache & Auger, 2003; Franken *et al.*, 2003; Henkel *et al.*, 2008; Leushuis *et al.*, 2010; Mallidis *et al.*, 2012; Menkveld, 2013). The procedure, the type of classification used (strict criteria, modified David classification, traditional or liberal approach, etc.) and the sperm morphology assessment reading technique are still very much under debate (Auger, 2010; Eliasson, 2010; Menkveld *et al.*, 2011). The lack of technical standardization and the subjective nature of the assessment make it difficult to compare WHO values and inter-laboratory values (Menkveld *et al.*, 2011). Internal quality control (IQC) monitors precision and external quality control (EQC) monitors the accuracy and stability of the methods, so both are important complementary processes to assess the analytical reliability of the technique.

Eustache and Auger have demonstrated wide inter-operator variability in France in the assessment of the percentage of normal forms and the detail of abnormalities according to the modified David classification (Eustache & Auger, 2003): during an external quality assessment program using projected images, the CV for the percentage of normal forms was 40% with a percentage of normal forms ranging from 6 to 39%. The most reliable CV was for the MAI (12%), acrosome abnormalities (26%), misaligned midpieces (23%), and absent tails (25%). Some abnormalities reach extremely high CVs (thin head 72%, thin midpiece 114%, short tail 145%). Other authors reported strong inter-operator variability (CV between 21 and 65%) (Davis & Gravance, 1993; Matson, 1995; Ombelet *et al.*, 1998; Keel *et al.*, 2000; Wang *et al.*, 2014). These authors suggested that even with the strict criteria recommended by the latest manual, it is still difficult for evaluators to give a precise assessment, particularly for head defects. In another study (Wang *et al.*, 2014), the CV for the various abnormalities ranged from 4.80% (irregular caliber) to 132.97% (thin midpiece), and the coefficients of agreement (kappa test) for specific defects such as head defects, asymmetrical midpiece, or coiled-in tail were all <0.40 and were considered fair or slight. For some types of defect, such as 'tapered', 'pyriform', and 'amorphous' spermatozoa, agreement was low: tapered (0.067; 0.243; 0.029), pyriform (0.134; 0.303; 0.199), and amorphous (0.061; 0.306; 0.084). The authors concluded that the main reason for such poor agreements is that the criteria of these defects are abstract and difficult to grasp.

Evidently, tightening the rules for defining a normal spermatozoon will affect the result. In a study of 8846 men, van den Hoven *et al.* highlighted a dramatic decrease in the percentage of normal forms between 1986 and 2011, from 30–80% to 0–10% related to changes in the assessment methodology (corresponding to the different versions of the WHO manual from 1980 to the strict criteria of 2010) (van den Hoven *et al.*, 2015). However, according to a Chinese study, the precisely defined criteria for sperm morphology normalcy in the 5th edition of the WHO

laboratory manual are less strict than the more subjective criteria of the 4th edition. This is shown in the same population by the significantly higher percentage of normal forms according to the 5th edition (26.50 ± 5.06)% than to the 4th edition (11.39 ± 3.17)% ($p < 0.05$) (Zhang *et al.*, 2011a). Morbeck *et al.* (2011) found a relationship between sperm morphology and pregnancy rate in intra-uterine insemination (IUI) during the years 1996–1997, whereas this relationship was not present in 2005–2006 due to a marked difference in the use of the classification in their laboratory. This difference was related to changes in technicians, differences in training, lack of quality control and changes in international standards.

In a Chinese team (Yao *et al.*, 2010), regular training sessions for standardizing the assessment of morphological criteria reduced inter-individual variability: the average difference between three technicians decreased from 4.57% ± 3.69% to 1.96% ± 1.19% after training. Franken and Kruger (Franken & Kruger, 2006) showed that competency in reading sperm morphology could be acquired and maintained through training sessions, external quality control programs and during annual refresher courses. One interesting point was that only the technicians who underwent regular training (five out of 19 in this study) were able to maintain their competency for more than 40 months. The others showed a decline 6 to 9 months after the initial training.

In a Belgian national External quality Control EQC of semen analysis (Punjabi *et al.*, 2016), 87 smears for sperm morphology were distributed between 1998 and 2012 to about 120 participants each year. The median CV over this whole period was 79.4% (lower quartile 53.9, upper quartile 94.4). Even if CV improved over the years with increasing use of WHO recommendations, the CV remains very high for sperm morphology (between about 40 and 90% in 2012). The results of the Belgian EQC program revealed large variability for sperm morphology and much more acceptable variability for other semen analyses: 19.2% for sperm count, 15.1% for progressive motility. Another report of the results of EQC in a program performed in 71 laboratories in the Tuscany region (Italy) showed a huge variability in the procedures and the results (Filimberti *et al.*, 2013). The highest variability was found for morphology (CV above 80% for all the trials, range 88.6 – 105.6%). In this survey, sperm morphology training courses made it possible to decrease variability to some extent, but CVs still remained >50%, which is not acceptable.

Evaluation of the accuracy of the test by EQC is difficult even within a same group of peers (same technique), due to the inter-laboratory variations mentioned. Therefore, we suggest that each laboratory must regularly monitor the average value obtained on its own samples for percentage of normal forms in order to detect any deviation over time, because of difficulties in interpreting EQC results.

Surprisingly, automated reading is not yet very widespread. In automated reading, the operator's influence on the assessment of studied cells is reduced; however, some CASA systems require an intervention to select cells for study. Additionally, automated systems also help to archive data with images and videos that are excellent materials for training staff. These systems seem to offer better intra- and inter-individual reproducibility (Menkveld & Kruger, 1995; Marnet *et al.*, 2000) even if intra- and inter-operator variability problems have already been reported on the first automated systems. In the study of Menkveld *et al.* (Menkveld *et al.*, 1997), the coefficients of variation (CV) were very high (from 40 to 60%) not only for manual evaluation but also for automated evaluation. So although automated evaluation is simpler than manual assessment, training and quality control on automated systems should not be neglected.

Staining technique: an important technical aspect

Before reading under a microscope, smear fixation and staining are mandatory. There is still considerable heterogeneity in carrying out preparation, staining and smear reading techniques. It is primordial to use staining techniques recommended by the WHO (Papanicolaou, Diff-Quik and Shorr). Since osmolarity varies from one stain to another, cell size also varies as a function of the technique used (van den Hoven *et al.*, 2015). Mortimer and Menkveld (Mortimer & Menkveld, 2001) recommend Papanicolaou stain for the best morphological assessment. The WHO manual also accepts the use of Shorr stain and Diff-Quik stain. The latter techniques have the advantage of being fast, but they provide fewer details of spermatozoon appearance than the Papanicolaou stain (Mortimer & Menkveld, 2001). However, no statistically significant difference has been observed when comparing Papanicolaou and Diff-Quik (Kruger *et al.*, 1987a; Menkveld *et al.*, 1997). One study showed very different results between the Diff-Quik and TestSimplets methods (TestSimplets is a quick staining procedure, without a fixation step and using a wet mount on special slides) (Natali *et al.*, 2013) This highlighted the importance of using the same stains for inter-laboratory comparisons, for application in decision-making thresholds in ART or even for the proper use of reference values published in the literature.

Pathophysiology of morphological abnormalities

The origin and impact of some morphological abnormalities remain unknown, possibly because there is a physiological element in the development of most of these abnormalities. However, some sperm morphology defects may be associated with functional abnormalities such as changes in chromatin condensation, defects in the acrosome reaction, problems with tail motility or even an increase in phenomena of apoptosis or necrosis (Menkveld *et al.*, 1990; Bastiaan *et al.*, 2003; Abu Hassan Abu *et al.*, 2012; Franken, 2015). There are also some specific defects (affecting 99 or 100% of spermatozoa) associated with genetic abnormalities such as globozoospermia, sperm macrocephaly syndrome, multiple tail abnormalities, or headless spermatozoa.

Relationship between the percentage of normal forms and functional abnormalities

Passage through the mucosa. Gneist *et al.* (2007) highlighted that there was reduced glycodelin binding to normal-shaped male gametes compared with abnormal spermatozoa (strict criteria). Glycodelin is a seminal plasma protein which may play a role in the suppression of capacitation. Decreased glycodelin adherence may facilitate the passage of spermatozoa through cervical mucus during natural conception (Gneist *et al.*, 2007).

Binding to the zona pellucida and acrosome reaction. Abnormal spermatozoa appear to have reduced ability to undergo

acrosome reaction in response to binding to the zona pellucida (Liu & Baker, 1994) or binding induced by calcium ionophore (Liu & Baker, 1998).

The percentage of normal forms has been found to be significantly correlated with the percentage of living spermatozoa that have undergone acrosome reaction induced by binding to the zona pellucida ($r = 0.518$; $p < 0.0001$; $n = 92$) (Abu Hassan Abu *et al.*, 2012), and to be inversely correlated with spontaneous acrosome reaction rate (Parinaud *et al.*, 1995). A significant correlation has been shown between the percentage of normal forms and the ability to fertilize hamster oocytes (Bronson *et al.*, 2007).

Despite these negative correlations demonstrated between morphology and functional abnormalities, some authors consider that morphology has a low predictive value for these functional abnormalities (Bronson, 2016). Spermatozoa exhibiting <5% of normal forms are in fact still able to effectively penetrate zona free hamster oocytes (>95% of penetrated oocytes, (Bronson *et al.*, 2007).

Relationship between the percentage of normal forms and DNA integrity or chromatin quality

The percentage of normal forms has been significantly inversely correlated to chromatin condensation by chromomycin A3 ($r = -0.745$; $p < 0.0001$; $n = 92$) (Abu Hassan Abu *et al.*, 2012).

Correlation between the percentage of normal spermatozoa and chromatin quality has also been demonstrated by other authors (Franken *et al.*, 1999; Esterhuizen *et al.*, 2000), but refuted by others (Beletti & Mello, 2004). In infertile patients, polymorphism of the protamine 1 (PRM1) promoter gene is associated with an increase in the protamine 1/protamine 2 ratio and an alteration in sperm morphology (<9% Kruger), probably related to the change in chromatin conformation (Gazquez *et al.*, 2008).

A significantly increased rate of disomic sperm evaluated by FISH (X, Y, 8 probes) was found in 70 patients with isolated teratozoospermia (<20% David) compared with 30 fertile controls, with a correlation with head abnormalities: amorphous shaped sperm were positively correlated with disomy X ($r = 0.377$, $p = 0.01$) and total sex chromosome aneuploidy ($r = 0.310$, $p < 0.05$), short tails were slightly but significantly correlated with the incidence of disomy X ($r = 0.293$, $p < 0.05$), and disomy XY ($r = 0.287$, $p < 0.05$) (Brahem *et al.*, 2011). In another study, the same team highlighted a significant increase in the rate of disomy 18, X and Y in 30 patients with severe polymorphic teratozoospermia (<20% David) compared with a group of 15 controls with normal sperm parameters. Count and motility also significantly differed between the two groups in this study (Mehdi *et al.*, 2012). However, a review and meta-analysis (Sun *et al.*, 2006) indicated that FISH studies did not show a specific association between morphology and chromosomal abnormalities, except in rare cases of monomorphic teratozoospermia such as macrocephalic sperm syndrome. Studies in this meta-analysis indicated that, like other forms of semen alteration (oligozoospermia or asthenozoospermia), teratozoospermia is associated with a very modest increase in chromosomal abnormalities. In this review, studies found that aneuploidy in men with teratozoospermia and asthenoteratozoospermia was 2 to 3-fold higher than in normal controls. (Ushijima *et al.*, 2000) reported that the oligoasthenoteratozoospermia group showed a very moderate increase in frequency of disomy for chromosomes 13 (0.13% vs. 0.09%; $p < 0.001$), 21 (0.24% vs. 0.19%; $p < 0.05$), sex (0.59% vs. 0.38%; $p < 0.001$), and diploidy (0.29% vs. 0.16%; $p < 0.005$) compared with the control group. These correlations between sperm morphology and aneuploidy, sometimes found in ejaculated sperm, are not found after fertilization. Karyotype data from spermatozoa after penetration in hamster oocytes do not show any relationship between chromosomal abnormalities and sperm morphology in fertile men and in men carrying translocations (Amelar *et al.*, 1973; Balkan & Martin, 1983; Martin, 1984; Martin *et al.*, 1987; Sun *et al.*, 2006).

Concerning DNA fragmentation abnormalities, Brahem *et al.* (2011) also reported a significantly increased rate of fragmented DNA measured using TUNEL assay in their teratozoospermic population compared with a fertile population. This is not in agreement with results obtained by other teams, who found no clear relationship between sperm morphology and the degree of DNA damage (Evenson *et al.*, 1999; Donnelly *et al.*, 2001; Trisini *et al.*, 2004). Trisini *et al.* and Donnelly *et al.* found no correlation between either the percentage of normal forms (strict criteria) and DNA integrity (Comet assay) or between specific head abnormalities (deformed head, macrocephaly) and DNA integrity. Evenson *et al.* found only a very weak correlation ($r = 0.21$ to 0.31; $p < 0.05$) between sperm chromatin structure assay (SCSA) and morphology (strict criteria).

A recent work (Jenkins *et al.*, 2016) has investigated a possible association between different sperm parameters (which included sperm head morphology) and DNA methylation markers in mature spermatozoa. These authors found no difference in overall methylation and regional methylation profiles for specific genes subject to parental imprint between the normal morphology group (>30% normal heads) and the group with <30% of normal heads.

Pathophysiology of certain abnormalities

Head abnormalities

Microcephalic heads. These are defined as a head <3.5 μm in length and 2.5 μm in width and are often associated with acrosome abnormalities (Menkveld *et al.*, 2011). In in vitro fertilization (IVF) and even in intra-cytoplasmic sperm injection (ICSI), fertilization rates are low but can be improved by more stringent selection of spermatozoa before micro-injection (Kihaile *et al.*, 2003) (selection of spermatozoa with a more oval head during an ICSI procedure). Gandini *et al.* (2000) highlighted a correlation between DNA fragmentation using TUNEL and the presence of microcephalic heads.

Total globozoospermia syndrome. Globozoospermia (spermatozoa with round heads and no acrosome) is a rare syndrome (incidence <0.1% of infertile men) responsible for male infertility (Holstein *et al.*, 1973). The presence of 100% globozoospermatozoa in the ejaculate defines the total syndrome. A combination of several defective mechanisms may be involved in the formation of the spermatozoon during spermiogenesis and may lead to lack of acrosome formation, which is sometimes associated with cytoskeletal abnormalities (Longo *et al.*, 1987). Over the last three decades, the etiology of these reported cases is still unclear but genetic causes have been found, notably in families with

globozoospermic brothers. In men, the first identified mutation responsible for total globozoospermia was a homozygous mutation of the SPATA 16 gene (Dam *et al.*, 2007). This gene is expressed in testicular tissues, and it encodes for a fusion protein whose expression was located using immunofluorescence on the Golgi apparatus and on the pro-acrosomal vesicle in the spermatids, giving rise to speculation of a crucial role during acrosome formation during spermiogenesis. In 2011, Koscinski *et al.* (2011) identified the DPY19L2 deletion as a major cause of globozoospermia. This deletion of 200 kb encompasses only gene DPY19L2 on chromosome 12. The frequency of heterozygous deletion is evaluated at 1/222. In human spermatozoa, it has a role in acrosome formation and elongation of the spermatozoa. Three recent studies confirmed the high prevalence of DPY19L2 gene alterations in patients with globozoospermia from different ethnicities and geographical origins (Coutton *et al.*, 2012; Elinati *et al.*, 2012; Zhu *et al.*, 2013). Homozygous deletions represent 26.7 to 73.3% of DPY19L2 mutations. The fertilization rate even after ICSI remained low. Oocyte activation (in particular with calcium ionophore) could improve the pregnancy rate significantly when dealing with globozoospermia (Chansel-Debordeaux *et al.*, 2015).

Thin (modified David classification) or elongated heads (strict criteria). A increasing incidence of thin heads, from 2 to 10%, was reported during experimental scrotal hyperthermia of 6 to 24 months duration (Mieusset *et al.*, 1987). A similar finding was also observed in varicocele with a decrease from 14% to 6.4% after embolization ($p < 0.01$) (Prasivoravong *et al.*, 2014). Elongated heads are often associated with tail insertion abnormalities, residual cytoplasmic material and aneuploidies (Prisant *et al.*, 2007).

Macrocephaly. Patients with macrocephalic sperm syndrome have 100% macrocephalic spermatozoa, with large irregular heads, sometimes an abnormal midpiece and acrosome, and multiple tails (on average, 3.6 per head). They present primary infertility. Ultrastructural study revealed a significant 3-fold increase in nuclear volume (Escalier, 1983). Teratozoospermia is generally associated with asthenozoospermia. A high rate of aneuploidy and polyploidy has been reported in patients with this syndrome (Achard *et al.*, 2007; Perrin *et al.*, 2008; Coutton *et al.*, 2015). It is likely that this syndrome results from nondisjunction of the chromosomes or defective cytokinesis during the first or second mitotic division. Homozygous mutations have been identified in the central part of the distal region of the long arm of chromosome 19: the AURKC gene (Dieterich *et al.*, 2009; El Kerch *et al.*, 2011; Ben Khelifa *et al.*, 2014; Eloualid *et al.*, 2014; Ounis *et al.*, 2015). This gene is involved in chromosome segregation and cytokinesis during spermatogenesis. AURKC is part of the Aurora kinase family that has a key role in the control of mitosis and meiosis (Coutton *et al.*, 2015). AURKC is predominantly expressed in the testicles. The c.144delC deletion leads to the synthesis of a truncated protein and is found in about 85% of mutated alleles (Ben Khelifa *et al.*, 2014). The prevalence of this mutation in the heterozygous state is particularly high in the Maghrebian population (1/50) (Dieterich *et al.*, 2009). There may be mosaic forms with a variable rate of macrocephalic sperm and a lower rate of aneuploidy in these situations. Where 100% of spermatozoa are affected by an identified AURKC mutation, ICSI is ineffective and contraindicated, and sperm donation may be suggested to the couple (Perrin *et al.*, 2008).

Cases of macrocephaly (aside from the previously described genetic syndrome) occur after treatment with sulfasalazine for ulcerative colitis and Crohn's disease. The abnormality may disappear when treatment is discontinued or if sulfasalazine is replaced by mesalazine (Toth, 1979; Cosentino *et al.*, 1984).

Headless spermatozoa and non-inserted tail defects

These abnormalities are also called decapitated sperm syndrome or 'pin heads', and have not been widely studied. They arise from a defect in distal centriole migration during spermiogenesis. The resulting phenotype has no head, a head-midpiece attachment defect, or both. To date, no genetic abnormality has been identified. Micro-injection of spermatozoa with a head-midpiece attachment defect leads to fertilization which is not followed by pro-nuclear fusion or cleavage (Chemes *et al.*, 1987, 1999).

Residual cytoplasmic material

Some authors differentiate cytoplasmic droplets (normal events) from excess residual cytoplasm, i.e. when cytoplasmic residue is present around the spermatozoon midpiece in excessive quantity (Rengan *et al.*, 2012). Normally, a human spermatozoon retains a small cytoplasmic droplet around the midpiece after the spermiogenesis process. Physiological cytoplasmic droplets have functional significance and are involved in hyperactivation, capacitation and the acrosome reaction (Rengan *et al.*, 2012). Retention of excessive cytoplasm around the midpiece is due to incomplete cytoplasmic extrusion. In comparison with the typical cytoplasmic droplet found in normal ejaculated human spermatozoa, excess residual cytoplasm contains elevated levels of cytoplasmic enzymes that produce pathological quantities of reactive oxygen species (ROS) (Gomez *et al.*, 1996; Rengan *et al.*, 2012). Excess residual cytoplasm is more abundant: its area is 30% greater than the sperm head area. The incidence of excess residual cytoplasm (not including physiological cytoplasmic droplets) is very low in fertile men (Auger *et al.*, 2016).

Tail abnormalities

These abnormalities systematically lead to asthenozoospermia. There are various abnormalities (absent, short, angular or irregular tails) and they are sometimes combined with spermatozoa head abnormalities.

In 1977, Eliasson *et al.* highlighted that a congenital defect of the cilia combined with a dynein defect in the tail is the cause of chronic respiratory infections and male sterility. Three of the six patients studied had situs inversus, corresponding to Kartagener syndrome (Eliasson *et al.*, 1977). It was noteworthy that the tails appeared normal under optical microscopy despite functional disorders (immobility).

Thick tails or irregular tails are sometimes associated with periaxonemal abnormalities (Feneux *et al.*, 1985). Other authors highlighted correlations between certain tail characteristics evaluated by optical microscopy and axonemal anomalies demonstrated by electron microscopy (Mitchell *et al.*, 2015).

The various flagellar anomaly phenotypes associated with genetic mutations have already been described as 'short tails', 'stump tails' or 'dysplasia of the fibrous sheath' (Neugebauer

et al., 1990; Chemes *et al.*, 1998; Davila Garza & Patrizio, 2013). Ben Khelifa *et al.* (2014) combined this heterogeneous group under the term of multiple morphological abnormalities of the sperm flagella. (Ben Khelifa *et al.*, 2014). Genetic abnormalities have been identified leading to phenotypes including angled tails and/or acrosome abnormalities, such as mutations of the solute carrier family 26 (SLC26A8) or septin 12 (SEPT12) (Kuo *et al.*, 2012; Dirami *et al.*, 2013; Coutton *et al.*, 2015). Among patients presenting a more serious phenotype with 100% tail abnormalities, 28% had a mutation on the DNAH1 gene, which encodes for an inner dynein heavy chain. This mutation may be associated with general axonemal disorganization, including mislocalization of microtubule doublets and loss of the inner dynein arms (Ben Khelifa *et al.*, 2014).

The percentage of coiled spermatozoa (not counting the heads in the coil) has a very low correlation with age ($r = 0.26$; $p = 0.003$) and epididymal alpha-glucosidase ($r = 0.016$; $p < 0.01$). Coiled spermatozoa are associated with heavy smoking and varicocele (Yeung *et al.*, 2009).

The level of expression of TCP11 (human y-complex protein 11) is 70% less in samples containing high rates of spermatozoa with coiled tails (Liu *et al.*, 2011). Their results show that TCP11 interacts with ODF1 (a major component of outer dense fibers) that plays a role in flagellar morphogenesis.

Morphological abnormalities and exposure to specific environmental factors or clinical contexts: tobacco and cannabis, temperature, obesity, testicular cancer, varicocele, urogenital infections

Tobacco and cannabis

Most studies on this topic showed that tobacco consumption has little impact on sperm morphology (Hoidas *et al.*, 1985; Pacey *et al.*, 2014), although they varied in their methods for assessing sperm morphology (Jeng *et al.*, 2014). Pacey *et al.* (2014) showed that men aged ≤30 years who used cannabis in the 3 months prior to sample collection were more likely to have sperm morphology <4% normal forms (strict criteria, WHO 2010) (OR = 1.94, 95% CI 1.05–3.60). These authors hypothesized that the cannabinoid receptor pathway had an impact on chromatin remodeling, as in mouse spermatids (Chioccarelli *et al.*, 2010).

Polychlorinated biphenyls (PCBs)

Men exposed to PCBs and dibenzofurans have alterations in sperm morphology (Hsu *et al.*, 2003). The percentage of abnormal forms (WHO 1992 criteria) was increased in the group exposed to PCBs compared with the non-exposed group (27.5 vs. 23.3%; $p = 0.04$).

Temperature

MacLeod & Gold (1951)found a significant decrease in sperm motility and sperm morphology in medical students after a febrile event (chickenpox and pneumonia), with recovery 4 weeks later. For Carlsen *et al.* (2003), the percentage of normal forms was reduced by 7.4% (± 11.6 ± 3.0) after a febrile event during the post-meiotic period of spermatogenesis (spermiogenesis). A regular and significant increase in abnormal forms (from <30% to more than 50%) and of some abnormalities (mainly thin heads) has been reported during experimental scrotal hyperthermia (Mieusset *et al.*, 1987). Mieusset *et al.* observed that recovery began a few days after hyperthermia was discontinued. This suggests that temperature has an impact on remodeling of the spermatozoon head during spermiogenesis and epididymal transit. However, the percentage of normal forms did not return to its baseline level until 8 months after discontinuation of exposure, and a longer period was necessary for some abnormalities of the midpiece and tail. The impact of temperature is complex and probably also affects the first stages of spermatogenesis. In 1992, a Danish study found a significant decrease in normal forms in 17 welders who were exposed to radiant heat for 6 weeks compared with a control group of 73 unexposed welders (Bonde, 1992). Another study showed a decrease in normal forms in semen samples from Roman taxi drivers compared with a group of 50 controls matched for age and tobacco use (45.8% vs. 64.0%, $p < 0.05$) (Figa-Talamanca *et al.*, 1996). However, we must consider the possible influence of other risk factors in such studies.

Some authors have observed that the season affects sperm morphology (Zhang *et al.*, 2013; Pacey *et al.*, 2014), but not others (Gyllenborg *et al.*, 1999; Jorgensen *et al.*, 2001).

Obesity

No relationship was found between obesity and sperm morphology (Pacey *et al.*, 2014; Eisenberg *et al.*, 2015) although an increase of time-to-pregnancy has been observed in couples whose BMI was ≥35 kg/m^2 compared with leaner couples (BMI <25 kg/m^2) (Sundaram *et al.*, 2017).

Testicular cancer

According to a recent study, patients with testicular cancer show an increase in some abnormalities: microcephalic spermatozoa, abnormalities of the post-acrosomal region, acrosomal defects, excessive residual cytoplasm and short tails (Auger *et al.*, 2016). It still remains unclear how this disease interferes with sperm morphogenesis. Several mechanisms seem to be involved. Moreover, fever during the disease could also have an impact. Patients with testicular cancer present oligoasthenoteratozoospermia before any treatment (Rives *et al.*, 2012). Alteration of sperm quality before any treatment, is due to the effect of the tumor itself or because testicular germ cell tumors is more frequent in men with other testicular disease (Giwercman *et al.*, 1989; Moller *et al.*, 1996; Horwich *et al.*, 2006; Bujan *et al.*, 2013). Several explanations have been proposed for sperm alteration before cancer treatment, such as cryptorchidism (a well documented risk factor). However, other causes could be suggested, such as tumor-associated secreted factors or stress (Bujan *et al.*, 2013). Moreover, testicular germ cell tumors are linked in part to the testicular dysgenesis syndrome (Skakkebaek *et al.*, 2001), which also leads to defective spermatogenesis.

Varicocele

Sperm morphology assessment (Kruger) revealed a decrease in normal forms in patients with varicocele (Zumrutbas *et al.*, 2013). In another study (Yeung *et al.*, 2009), 43 men with varicocele had a significantly higher rate of coiled tails compared with 384 patients without varicocele (mean ± SEM 10.8 ± 0.9% vs. 9.0 ± 0.3%, respectively, $p = 0.027$). This type of result is difficult to interpret, as it depends on the analytical reliability of the

technique (see section 4 *Analytical performances of sperm morphology assessment*).

Urogenital infections

Zhang *et al.* (Zhang *et al.*, 2011b) showed a significant decrease in the percentage of normal forms in Ureaplasma urealyticum-positive sperm samples compared with negative samples and with fertile controls. These results are not in agreement with previous work (Sanocka-Maciejewska *et al.*, 2005). Moreover, in the study of Zhang *et al.*, there are no data on conventional sperm parameters other than sperm morphology.

Question on the clinical value of assessment of the frequency of each detailed abnormality in addition to the percentage of normal forms

Sperm abnormalities are sometimes correlated with particular clinical situations, but with a very moderate sensitivity and specificity (see section 4). The question thus arises of whether it is clinically relevant to systematically evaluate specific abnormalities in fertility check-ups. Most laboratories systematically count the different abnormalities, while 47.7% of clinicians interviewed do not take them into consideration (Gatimel *et al.*, 2016). This is probably due to the very moderate clinical relevance of these different abnormalities, apart from the rare monomorphic abnormalities syndromes. The frequency of each morphological abnormality is significantly higher in infertile men than in fertile men (Auger *et al.*, 2016). The latter is the first report on the distribution of the different abnormalities of the head, the midpiece and the tail in a group of fertile men. The WHO 2010 manual does not recommend systematic detailing of abnormalities. Furthermore, the major problem with the assessment of these abnormalities is the very wide range of intra- and inter-operator variability (Eustache & Auger, 2003; Wang *et al.*, 2014) (see section 4 *Analytical performances of the sperm morphology assessment*). This is unacceptable given the current recommendations in terms of quality assurance to ensure the reliability of analyses (ISO 15189 standard (Vassault, 2013)).

PROGNOSTIC VALUE OF SPERM MORPHOLOGY ASSESSMENT BEFORE ART

Impact of sperm morphology on intra-uterine insemination (IUI) outcomes

It has been shown that the results of IUI are strongly dependent, where male parameters are concerned, on the number of motile spermatozoa after preparation (Monraisin *et al.*, 2016). The minimum threshold for number of motile spermatozoa after preparation recommended for inseminations is 1 million (WHO recommendations, 2010). There is no consensus for morphology. The impact of the percentage of normal forms (NF) on the rate of pregnancy by intrauterine insemination (IUI) is still debated (Table 2).

Before 2011, some studies (Hauser *et al.*, 2001; Van Waart *et al.*, 2001; Lee *et al.*, 2002; Spiessens *et al.*, 2003; Shibahara *et al.*, 2004; Grigoriou *et al.*, 2005; Badawy *et al.*, 2009; Nikbakht & Saharkhiz, 2011) showed that the rate of pregnancy by IUI was higher for a NF value greater than a defined threshold (most often 4% using strict criteria). However, other studies (Karabinus & Gelety, 1997; Check *et al.*, 2002) found no significant impact of morphology on pregnancy rate. A review and meta-analysis (Van Waart *et al.*, 2001) included 18 original articles, of which 6 were statistically analyzed. These 6 studies used the Tygerberg strict criteria. This meta-analysis concluded on 'a significant improvement in pregnancy rate above the 4% threshold for strict criteria'. In this meta-analysis, the threshold values showing an impact of spermatozoa morphology on pregnancy rates was 4% in most cases when the strict criteria were used and ranged from 8% (Comhaire *et al.*, 1995) to 50% (Francavilla *et al.*, 1990) when the WHO criteria (1987, 1992) were used. However, most of these studies did not concern isolated teratozoospermia, and important male and female characteristics were lacking in the publications included in the meta-analysis.

The study of Ombelet *et al.* (Ombelet *et al.*, 1997c) showed an impact of morphology on pregnancy rates after IUI only when the number of motile spermatozoa after preparation was <1 million, a threshold under which IUI is not recommended in any circumstances.

Recent publications on the subject found no significant difference in pregnancy rates in IUI cycles between groups with or without isolated teratozoospermia (Sun *et al.*, 2012; Deveneau *et al.*, 2014; Lockwood *et al.*, 2015; Lemmens *et al.*, 2016). In their retrospective study, (Deveneau *et al.*, 2014) included not only male infertility but also female causes of infertility (ovulatory in particular). Confounding factors were examined by multivariate analysis. These variables included history of pregnancy, the age of the two members of the couple, the number of cycles, the type of cycle (natural or stimulated), and the various infertility diagnoses. This study showed that the number of motile spermatozoa after preparation is the most significant factor influencing the rate of pregnancy and that morphology should not affect indications of IUI.

Contradictory results in the literature are probably linked to the wide range of intra- and inter-laboratory variations in morphology assessment by technicians, differences in staining methods, in the way teratozoospermia is defined (<5%, <15%, etc.) and of course, in the characteristics of the studied population: duration of infertility, female factors (age, ovarian reserve), cause of infertility. The progress of ART techniques has led to a change in the characteristics of the population treated by IUI.

In a prospective study (Erdem *et al.*, 2016), the percentage of normal forms (before and after preparation) was significantly higher in patients who achieved a live birth, but only in the male infertility subgroup and not in the unexplained infertility group.

In a retrospective study of 1166 couples and 4251 cycles, Lemmens *et al.* (2016) found that sperm parameters (WHO 5th edition criteria) had no predictive value for the likelihood of pregnancy. In this same study, a multivariate model showed, quite strikingly, that ongoing pregnancy rates were moderately negatively influenced by a percentage of normal forms >4% (Lemmens *et al.*, 2016).

Impact of sperm morphology on conventional in vitro fertilization (IVF) outcomes

In 1986, Kruger *et al.* described a sperm morphology assessment method known as the Kruger/Tygerberg criteria as a predictive factor for conventional IVF success (fertilization and pregnancy rates). Chances of success were low when normal forms (NF) were between 0 and 4%, intermediate when NF were between 5 and 14% and normal when NF >14% (Kruger *et al.*, 1986). In 1998, a structured literature review (Coetzee *et al.*,

Table 2 Summary of studies evaluating the impact of the percentage of normal forms on pregnancy rates after IUI

Authors	Number of IUI cycles (couples)	Methods	Classification (staining technique)	Impact of morphology on pregnancy rates	Comments
Van Waart *et al.* (2001)	2663 cycles	Metaanalysis of 18 original articles, of which 6 were statistically analyzed (Matorras *et al.*, 1995; Toner *et al.*, 1995; Lindheim *et al.*, 1996; Karabinus & Gelety, 1997; Ombelet *et al.*, 1997a,b,c; Montanaro-Gauci *et al.*, 2001)	WHO strict criteria 1987 and 1992 (unspecified)	Yes	Morphology >4% is associated with an improvement in PR: 2 studies showed an impact of sperm morphology, 3 a trend and one no impact.
Hauser *et al.* (2001)	264 cycles (108)	Retrospective	Kruger strict criteria 1986 (unspecified)	Yes	Only male infertility. PR decreaseed significantly (11.1% vs. 36.1% vs. 50.0%) NF ≤4%; 4–14%; >14%
Check *et al.* (2002)	412 first IUI cycles	Retrospective	Strict criteria (unspecified)	No	No significant difference in PR: 30% (28/91) for 0–4% NF, 26% (71/268) for 5–14%, and 20% (11/53) for >14%.
Lee *et al.* (2002)	244 cycles (209)	Retrospective	Kruger 1986 (Diff-Quik)	Yes	Significant difference on PR (3.8%; 18.5%; and 29.9%) for ≤4%; 4–9%; > 9% NF. Other sperm parameters normal
Spiessens *et al.* (2003)	872 cycles (440)	Retrospective	WHO strict criteria 1999 (Papanicolaou)	Yes	Predictive value of morphology <10% on cumulative PR (33% vs. 53% after 4 attempts) Other sperm parameters normal
Shibahara *et al.* (2004)	682 cycles (160)	Retrospective	CASA strict criteria (Diff-Quik)	Yes	Multivariate analysis, NF ≥15.5% [odds ratio (OR) = 2.2; $p = 0.02$] is predictive of the chances of pregnancy
Grigoriou *et al.* (2005)	1641 cycles (615)	Retrospective	Strict criteria (Papanicolaou)	Yes	Cumulative rates of live births significantly lower in teratozoospermia < 10% Other sperm parameters normal
Badawy *et al.* (2009)	714 cycles (393)	Prospective	David (Schorr)	Yes	Threshold < 30%, impact of morphology when the number of motile sperm after preparation is <5 million
Nikbakht & Saharkhiz (2011)	820 cycles (445)	Prospective	WHO strict criteria 1999 (unspecified)	Yes	The most marked difference was observed in comparing the morphology sub-groups <5% vs. 5–10% vs. >10% (2.1% vs. 10.1% vs. 12.6% PR, respectively)
Sun *et al.* (2012)	908 cycles (412)	Retrospective	WHO strict criteria 1999 (Papanicolaou)	No	No statistically significant difference in women under 35 years. Other l sperm parameters normal
Deveneau *et al.* (2014)	856 cycles (408)	Retrospective	WHO strict criteria 1999 (unspecified)	No	No significantly statistical difference for morphology with NF <4% compared to > 4% (17.3% vs. 16.7%; odds ratio 0.954, 95% confidence interval 0.66–1.37).
Lockwood *et al.* (2015)	70 cycles	Retrospective	WHO strict criteria 1999 (HEMA-3 stain)	No	Patients with isolated teratozoospermia <5%, pregnancy rates similar to those with normal morphology (15.7 vs. 13.9%)
Erdem *et al.* (2016)	442 cycles in the unexplained infertility group 88 cycles in the male infertility group	Prospective	WHO strict criteria 2010 (Papanicolaou)	Yes in the sub-group with masculine factors	NF (before and after preparation) was significantly higher in the group of patients who obtained a birth in the male infertility group, but not in the unexplained infertility group
Lemmens *et al.* (2016)	4251 cycles (1166)	Retrospective	WHO strict criteria 2010 (mix aniline blue/eosin)	No	Multivariate model showed that the rates of ongoing pregnancy were moderately negatively influenced by a % NF >4%

IUI, intra-uterine insemination; NF, normal forms; WHO, world health organization; CASA, computer-assisted sperm analysis; PR, pregnancy rate.

1998) showed that in more than 80% (a total of 18) of studies conducted between 1976 and 1996, the percentage of normal forms was positively associated with successful IVF (fertilization rate and clinical pregnancy rate) when the 5% or 14% threshold was used. One of these studies (Enginsu *et al.*, 1991) also found that the fact of correlating the morphology assessment to the sperm preparation test results offered a better predictive value for the likelihood of success for classic IVF. Thereafter, other studies confirmed the results of this meta-analysis and recommended ICSI below the 5% threshold for normal forms, regardless of the count and motility values (Marnet *et al.*, 2000; Gunalp *et al.*, 2001; Menkveld *et al.*, 2001). However, this strategy was undermined by several studies that did not find a significant decrease in the likelihood of pregnancy after IVF in the case of teratozoospermia (Ombelet *et al.*, 1994; Terriou *et al.*, 1997; Keegan *et al.*, 2007; Lundin, 2007).

Although many studies found that semen parameters other than morphology, motility (Hirsch *et al.*, 1986; Comhaire *et al.*, 1988; De Geyter *et al.*, 1992; Marnet *et al.*, 2000) or concentration (Liu & Baker, 1988; Biljan *et al.*, 1994) are also positively associated with fertilization and/or pregnancy, number of studies also showed that association between sperm morphology and IVF outcome was independent of any of the other semen parameters (Mahadevan & Trounson, 1984; Kruger *et al.*, 1986; Oehninger *et al.*, 1988; Liu & Baker, 1990; Grow *et al.*, 1994).

Most published studies found that teratozoospermia had an impact on conventional IVF outcomes.

Impact of sperm morphology on intra-cytoplasmic sperm injection (ICSI) outcomes

In practice, in cases of male infertility, the choice between IUI or IVF and ICSI is not very dependent on morphology because, on the one hand, there is no applicable recommendation, and on the other hand, teratozoospermia is very frequently associated with severe oligoasthenozoospermia (OATS), which is an immediate indication for ICSI. But if sperm parameters are only moderately altered, does isolated teratozoospermia justify recourse to ICSI? In 2011, a systematic review and meta-analysis (Hotaling *et al.*, 2011) studied the relationship between severe isolated teratozoospermia (<5% with the strict criteria) and clinical pregnancy rate after conventional IVF or ICSI through four retrospective studies (Lundin *et al.*, 1997; Osawa *et al.*, 1999; Keegan *et al.*, 2007; Dubey *et al.*, 2008). These studies provided precise data on conventional sperm parameters in the control group and the teratozoospermia group, with no statistically significant difference in the demographics of the men and women studied. They included 2853 IVF/ICSI cycles with 673 men with severe teratozoospermia and 2183 men without severe teratozoospermia. Teratozoospermia was not associated with lower clinical pregnancy rates either with IVF or with ICSI. Two of these four studies (Osawa *et al.*, 1999; Keegan *et al.*, 2007) concluded that the results of IVF/ICSI were not affected by severe teratozoospermia. (Dubey *et al.*, 2008) concluded that among couples treated by IVF (without ICSI), men with normal sperm parameters were much more likely to achieve a pregnancy than those with teratozoospermia (OR 3.19; 95% CI: 1.1 to 9.0). In a sub-analysis of IVF-ICSI couples, (Lundin *et al.*, 1997) suggested that severe teratozoospermia increased the risk of poor results more than three-fold compared with normal sperm (OR 3.36; 95% CI: 1.53 to 7.40) (OR 3.36; 95% CI: 1.53 to 7.40).

In a retrospective study on 332 ICSI cycles, Sariibrahim *et al.* (Sariibrahim *et al.*, 2013) found no statistically significant difference between their three groups defined according to Kruger strict criteria (<4%; 4–14%, >14%) with regard to the rates of fertilization, implantation, clinical pregnancy, and live births. In a retrospective study including 3922 conventional IVF and 843 ICSI procedures, Li *et al.* (Li *et al.*, 2014) demonstrated that the rate of fertilization decreased with the percentage of normal forms with strict criteria in conventional IVF but not in ICSI. In a recent study (van den Hoven *et al.*, 2015), the percentage of normal forms appeared to be correlated with ongoing pregnancy rates with an OR of 1.06 [1.02–1.16] in conventional IVF ($n = 2323$) but not in ICSI ($n = 1353$). A statistically significant relationship has been observed between a decrease in the percentage of normal forms and lower chances of ongoing pregnancy in conventional IVF, with, however, an area under the curve of only 0.54. The authors conclude that sperm morphology is not a good tool for predicting the ongoing chances of pregnancy through either IVF or ICSI. Another study compared the results after randomization between IVF or ICSI on sibling oocytes in a group of patients with isolated teratozoospermia (<4% strict criteria) ($n = 183$) and a group of patients with all normal sperm parameters ($n = 258$). They found no significant difference in fertilization rates, day 3 embryonic morphology, pregnancy and spontaneous abortion rates between the groups in either IVF or ICSI (Fan *et al.*, 2012).

Except for some specific severe teratozoospermia such as globozoospermia, no study has found that a total absence of normal forms has absolute predictive value for fertilization rate during IVF or ICSI. As shown by French *et al.*, 2010; sperm morphology is of low predictive value in ICSI: no ICSI outcomes (fertilization, rate, blastulation rate, implantation rate, pregnancy rate) were decreased in their subgroup with 0% of normal forms (French *et al.*, 2010).

Regarding ICSI, most publications agree that the percentage of normal forms is not predictive of either fertilization rates or pregnancy rates (Mansour *et al.*, 1995; Nagy *et al.*, 1995; Oehninger *et al.*, 1996; Svalander *et al.*, 1996; Lundin *et al.*, 1997; Sukcharoen *et al.*, 1998; Host *et al.*, 1999; Osawa *et al.*, 1999; McKenzie *et al.*, 2004; Keegan *et al.*, 2007; French *et al.*, 2010; Berger *et al.*, 2011). The main arguments explaining the lack of correlation between sperm abnormalities shown by sperm morphology assessment and the results of ICSI attempts are that the micro-injection makes it possible to bypass some of the obstacles encountered during natural processes, and that, during an ICSI attempt, the spermatozoon used for fertilization is not very representative of the whole population.

We must note the lack of any randomized controlled trials in the field, the large variations of the thresholds used between studies (even when the same classification is used) and the numerous biases of most retrospective studies. Using WHO 2010 recommendations, no studies are yet available on the value of sperm morphology assessment in IVF, and only two retrospective studies have been published for IUI with opposite results (Table 1). The decision to assign a couple to conventional IVF or ICSI must be based mainly on the number of total motile spermatozoa after selection.

CONCLUSION

For more than 30 years, numerous authors have endeavored to explain the pathophysiology of human sperm morphological abnormalities. They have highlighted correlations between the percentage of normal forms, some sperm functional abnormalities, and spontaneous fertility. The physiological nature of most morphological 'traits' of human spermatozoa makes sperm morphology very difficult to interpret. With the exception of the diagnosis of some very rare specific defects linked to genetic disorders (globozoospermia, macrocephaly, decapitated sperm syndrome, and fibrous sheath dysplasia) which could be easily done when assessing conventional sperm parameters on fresh sample, sperm morphology assessment has a very poor clinical impact not only in diagnostic investigation but also in the choice of ART technique. Moreover, since there are huge technical variations in morphology assessments from one laboratory to another, the published thresholds of normal sperm morphology used to assist in the choice of ART technique or to assess prognosis are not transposable. To be defined as a diagnostic test, sperm morphology assessment must fulfill, as other diagnostic tests, at least 3 successive criteria: analytic reliability, clinical validity and clinical utility. We have largely shown that sperm morphology assessment does not even fulfill the first criterion. Regarding its clinical validity defined as its ability to predict a clinical phenotype with good sensitivity and specificity, there are only very few studies reporting such values for sperm morphology assessment. Guzick *et al.* (2001) found no threshold of normal forms giving both sensitivity and specificity above 60%. Recently, a study argued that sperm morphology was of little clinical value, showing that men with a complete absence of normal sperm morphology exhibited high rates of spontaneous pregnancy without assisted reproduction (Kovac *et al.*, 2017). In this cohort of 24 men identified with 0% NF and 27 randomly selected men with ≥4% NF as controls, 29.2% of the men with 0% NF obtained a spontaneous pregnancy (controls = 55.6%, $p \leq 0.05$) after a median follow-up time of 2.5 years. Finally, clinical utility is estimated through prospective, randomized controlled trials in order to demonstrate the added value of the test on the clinical management or outcome. We cannot find real examples of studies to validate this criterion.

The lack of analytical reliability of this test is arises from heterogeneity in the preparation and reading of smears, lack of knowledge and homogeneity of classification systems, lack of measures to maintain technicians' and biologists' skills and, of course, the subjective nature of morphological assessment.

We suggest that, for laboratories who continue to perform sperm morphology assessment, it is becoming urgent and essential:

- to conduct internal and external quality control programs and regular refresher courses to maintain technicians' skills and biologists' knowledge,
- to regularly monitor the average value obtained by the laboratory for percentage of normal forms because of difficulties in interpreting EQC results,
- to end systematic determination of the frequency of each morphological abnormality in view of their clinical relevance and as they are sources of even very high variability. Such analysis should only be performed upon request during a specific andrological investigation.
- to discontinue the recording of any index of multiple defects (MAI, TZI, SDI) as there is very little evidence in the literature of its clinical interest.

Twenty years ago, Ombelet *et al.* (1997b) concluded that 'lack of standardization of sperm morphology assessments remains the main reason for the debatable usefulness of this parameter in the laboratory evaluation of semen'. We have to concede that this situation has not changed in 2017. For all these reasons, we seriously question the utility of systematic sperm morphology assessment whileanalytical problems are not completely resolved and until its clinical use is clearly codified through a consensus.

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CONFLICT OF INTEREST

None of the authors have conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

NG designed the study, collected data and drafted the manuscript JM, JP and RL read and helped to draft the manuscript. JP and RL helped to design the study. All authors read and approved the final manuscript.

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