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MEN'S HEALTH (A DABAJA, SECTION EDITOR)

Sperm Morphology: History, Challenges, and Impact on Natural and Assisted Fertility

Rachel B. Danis[1] · Mary K. Samplaski[2]

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Abstract

Purpose of Review The classification of morphologically normal sperm has been progressively redefined. Concurrently, our understanding of the significance of sperm morphology in relation to male factor infertility has evolved. In this review, we will discuss the evolution of sperm morphology assessment and factors that contribute to its measurement variability. We will examine the impact of sperm morphology on natural pregnancy, IUI, IVF, and ICSI outcomes.

Recent Findings There is a lack of consensus on sperm morphology classification, technique, and inter-observer grading variability. Current evidence suggests sperm morphology has low predictive value for pregnancy success, for both natural and assisted reproduction. Additionally, the threshold for what is considered an adequate percentage of morphologically normal sperm has changed over time. These variables have called into question the relevance of this variable in predicting fertility outcomes.

Summary Our understanding of the impact of sperm morphology on reproductive outcomes continues to evolve and seems to play less of a role than initially thought.

Keywords Sperm morphology · Semen analysis · Teratozoospermia · Infertility · Assisted reproductive technology · Male factor

Historical Significance

For years, sperm morphology has been a debated indicator of male fertility and success with assisted reproductive technologies (ARTs) [1]. While subfertile men have a lower percentage of normal forms when compared with men with proven fertility [2], the question of "Does form impact function?" remains. While an assessment of sperm morphology is a component of the standard semen analysis, the clinical utility of this is debated.

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✉ Rachel B. Danis
Rachel.danis@med.usc.edu

Mary K. Samplaski
mary.samplaski@med.usc.edu

[1] Division of Reproductive Endocrinology, University of Southern California, 2020 Zonal Avenue, IRD 534, Los Angeles, CA 90033, USA

[2] Institute of Urology, University of Southern California, 1441 Eastlake Avenue, Suite 7416, Los Angeles, CA 90089, USA

Examination of sperm that have passed through the cervical mucus have helped to define a "normal" shaped sperm [3, 4]. The concept of abnormal appearing sperm was first explored in the 1950s, when the individual morphologic abnormalities were described, and sperm without abnormalities were considered normal. This was used as the basis for the World Health Organization (WHO) 1st and 2nd editions [5, 6]. With the progressive description of sperm abnormalities, the WHO percentages for normal sperm decreased dramatically. In the 1st edition, the average normal morphology was 80.5% [5], which decreased to 50% in the 2nd edition [6], 30% in the 3rd edition [7], 14% in the 4th edition [8], and is currently 4% for the 5th edition [9].

For the 3rd edition of the WHO manual, the Tygerberg strict criteria were implemented. For these criteria, sperm with "borderline" abnormal features were classified as abnormal [3]. Because of this change, the number of sperm that were "normal" decreased dramatically (i.e., most sperm became morphologically abnormal). This detailed description of the criteria for a morphologically normal sperm is only found in the 3rd edition of the WHO manual, which provides a clear description of normal sperm with well-defined sperm head lengths and widths and qualitative

descriptions [7]. The 4th edition provided a list of various abnormalities [8], and the 5th edition provided a precision definition of normal sperm and different abnormalities along with schematic drawings [10]. The WHO currently recommends use of the strict morphology criteria to evaluate sperm morphology and has established 4% normal forms as the lower limit of normal [10].

The 2010 WHO manual defines a morphologically "normal" sperm as having a head (with acrosome), midpiece, and tail. Specifically, a "normal" head has an oval shape with smooth contours. The acrosome is clearly visible, well-defined, exhibits a homogenous light-blue staining, and covers 30–60% of the anterior portion of the sperm head. A "normal" midpiece lacks cytoplasmic residues and is axially attached to the head, without forming a definite angle with respect to the head, ≤ 1 μm in width and approximately 1.5 times the head length. The tail should also lack cytoplasmic residues, be apically inserted to the post-acrosomal end of the midpiece, have a length of approximately 45–50 μm long, and be lacking any sharp bends [11]. Sperm should be analyzed after being stained via a modified Papanicolaou method. The analyzer should assess at least 200 spermatozoa per sample [11].

In an attempt to circumvent the subjective nature of a visual assessment, computer-aided sperm analysis was developed. This system analyzes sperm kinetics in an attempt to provide more objective sperm parameters. While this computer-generated analysis attempts may minimize observer bias, it does not provide the detailed morphological assessment necessary to accurately define normal versus abnormal sperm, and therefore, may not be as useful for determining sperm morphology [12].

While it is commonly believed that the decline in reference values are mostly due to the introduction of strict criteria, there are some that believe there is an actual decline in the number of morphologically normal sperm due to environmental factors [13]. In addition, while a full discussion of the history and evolution of sperm morphology assessment systems is beyond the scope of this chapter, it is important to note that the use of other classification systems has had a major impact on the assessment of normal forms. Complicating this, the assessment of sperm morphology is still highly subjective and prone to inter- and intra-laboratory differences [14].

Of note, there are specific sperm morphology anomalies that do necessitate in vitro fertilization (IVF). These include globozoospermia, primary ciliary dyskinesia, and significant tail defects. These are sperm without the capacity to swim to the egg, or penetrate the egg. Conversely, macrocephalic heads have been associated with sperm aneuploidies, where intracytoplasmic sperm injection (ICSI) is ineffective and contraindicated [15]. The remainder of this review is dedicated to other, more heterogeneous, sperm morphologic abnormalities, and their impact on natural and assisted reproduction.

Sperm Morphology and Fertility

As sperm morphology assessment has evolved, the question has arisen, "If most sperm are characterized as abnormal, how can morphology be a reliable predictor of pregnancy success?"

The predictive value of sperm morphology was first proposed by Krueger et al., who found an inverse relationship between oocyte fertilization and sperm morphology [16, 17]. This was then reinforced by a Lancet study that found that men with men with decreasing sperm morphology had a lower likelihood of contributing to a pregnancy [18]. Likewise, it has been found that men who are part of a subfertile couple have a lower percentage of normal sperm, compared with men with proven fertility [2]. Conversely, later data showed that successful oocyte fertilization and pregnancies were reported even in couples with normal sperm morphology of 0% [19]. Subsequent data confirmed that even couples with no normal sperm have the ability to achieve pregnancy via natural means, intrauterine insemination (IUI), or IVF [20••]. Further complicating matters, a recent study found that many physicians believe that for men with normal morphology < 4%, natural means will not be successful in achieving a pregnancy [21••].

Natural Conception

There is scant data looking at the impact of sperm morphology on natural conception outcomes. In the only study looking at this parameter and outcome specifically, Kovac et al. conducted a retrospective chart review investigating the likelihood of achieving pregnancy without the use of ART in men with severe teratozoospermia, 0% normal forms, as per strict Kruger criteria [20••]. Twenty-four men with 0% normal forms were compared to 27 randomly selected men with ≥ 4% normal forms over a 3-year period. While the natural conception rate was higher in men with ≥ 4% normal forms compared to the severe teratozoospermia group (51.8% vs 25%, $p \leq 0.05$), men with 0% normal forms were still able to conceive naturally in 25% of cases. Additionally, in cases where men with 0% normal forms conceived naturally, 100% of these men had another child via natural conception. The authors concluded that strict morphology should not be used to predict fertilization, pregnancy, or live birth potential, and in men with 0% normal forms, alternative reproductive modalities should be considered before immediate IVF.

Intrauterine Insemination

As noted above, assessment of sperm morphology was historically done by looking at sperm present in the cervical mucus. But in ARTs which bypass the cervix, does sperm morphology impact outcomes? IUI success has been shown to be strongly

dependent on the number of total motile sperm post-wash [22]. According to the WHO 5th edition, the minimum threshold for number of motile sperm post-wash necessary for possible IUI success is one million [10]. However, there is no consensus for the percentage of morphologically normal sperm needed for IUI success.

Some early studies found that normal morphology > 4% was associated with pregnancy rates after IUI [23–25]. However, other studies found that sperm morphology did not impact pregnancy rates after IUI [26, 27]. A 1997 study found that that sperm morphology was only a prognostic factor for pregnancy when the inseminating motile count was < 1×10^6, and men with this threshold of motile sperm would not traditionally be considered a candidate for IUI regardless.

More recent publications have generally not shown a difference in IUI pregnancy rates in men with isolated teratozoospermia. In a retrospective study looking at the impact of sperm morphology on IUI success, which included both male and female factor causes for infertility, the post-wash motile sperm count was the most significant factor to influence pregnancy rates. In this study, sperm morphology did not change IUI success rates [28]. Other recent studies have had similar findings, specifically demonstrating a lack of correlation between normal morphology and IUI success [29, 30].

Conversely, a prospective 2016 study by Erdem et al. looking at the predictive value of sperm morphology for live births after IUI found for couples with known male factor infertility morphology did predict for live birth, but in couples with unexplained infertility it did not. In the male factor infertility group, a post-wash normal sperm morphology > 4.5% predicted for an increased probability of live birth [31••]. Similarly, a 2016 retrospective, observational study found mixed results for the impact of morphology on IUI success for ongoing pregnancy in couples treated with IUI (4251 cycles). Morphology was not predictive of pregnancy after the first IUI cycle. However, for couples undergoing multiple IUIs, sperm morphology < 4% was predictive for ongoing pregnancy [32].

More recently, Kohn et al. [21••] conducted a meta-analysis of 20 observational studies involving 41,018 IUI cycles looking at the impact of sperm morphology (> 4% and ≤ 4%, and ≥ 1% and < 1% normal forms) on ultrasound-verified pregnancy outcome [21••]. Using the WHO 3rd edition guidelines, a significant difference in pregnancy rates was seen when using the 4% normal morphology threshold. However, when using the WHO 4th or 5th guidelines (4% normal morphology threshold), no difference in pregnancy rates was seen (14.2% versus 12.1% versus 13.9% for normal forms > 4%, ≤ 4% or < 1%, respectively). Similarly, a recent randomized control trial found that in men with normal morphology < 4%, 3 cycles of stimulated IUI were as effective in achieving a pregnancy as one round of IVF, demonstrating that IUI can be as effective as IVF, even in men with teratozoospermia [33]. In conclusion, while earlier data were conflicting, the most recent data do not seem to show a strong correlation between sperm morphology and IUI success rates.

In Vitro Fertilization

Traditionally, strict sperm normal morphology was believed to be one of the "best predictors of IVF outcomes" [34]. In 1986, the Kruger/Tygerberg criteria for normal sperm morphology were shown to be predictive for IVF success (fertilization and pregnancy rates) in a progressive manner. If normal morphology was > 14%, there was a high chance of success. If normal morphology was 5–14%, intermediate chance of success, and if 0–5%, low chances of success [17]. Because of this, some have suggested that men with teratozoospermia, defined as < 5% normal morphology in a setting of otherwise normal semen parameters, undergo ICSI to improve pregnancy outcomes [35, 36].

However, in more recent years, the data on the effect of sperm morphology on IVF outcomes has been more heterogeneous. A 1998 structured literature review of 49 studies found that > 80% of published studies found that normal morphology did correlate with IVF outcomes [37]. Several follow-up studies confirmed these findings, which led to a generalized recommendation for ICSI for sperm morphology < 5% [38–40]. However, even for the older literature, this finding was not consistent. There were a number of studies showing no relationship between pregnancy rates after IVF and sperm morphology in men with isolated teratozoospermia [34, 41–43].

In conclusion, the most recent data looking at the impact of isolated teratozoospermia on outcomes of IVF outcomes does not seem to demonstrate a consistent relationship. This would suggest that the impact of sperm morphology on IVF outcomes is minimal or only in selected patients. However, despite this data, a 2017 study found that there is a prevailing belief among physicians that men with normal sperm morphology < 4% cannot reliably achieve a pregnancy except via IVF.

Intracytoplasmic Sperm Injection

Finally, does teratozoospermia impact ICSI success rates? Historically, isolated teratozoospermia was thought to compromise less invasive fertilization and pregnancy rates, which led investigators to examine the benefit of ICSI with IVF [16, 44]. The initial perception was that ICSI would "bypass" abnormal sperm morphology, as it compensates for many steps of sperm fertilization, including swimming to the oocyte (motility), binding to the zona pellucida, and the acrosome reaction [18, 45].

Originally, in the late 1990s, Pisarska et al. compared conventional (fresh) IVF versus IVF with ICSI with the use of

sibling oocytes from partners of subfertile males [46]. Investigators separately evaluated a subgroup of 20 subfertile males with only severe teratozoospermia (morphology ≤ 4% normal by Kruger's strict criteria) as the only identifiable cause for subfertility [46]. The authors found no differences in fertilization rates, number of fertilized oocytes, percentage of oocytes fertilized, and embryo quality for those undergoing IVF versus IVF with ICSI ($p > 0.05$). When the authors focused on the subgroup with severe teratozoospermia, there was a statistically significant difference between the number of oocytes fertilized and the percentage of oocytes fertilized per couple when comparing fresh IVF versus IVF with ICSI ($p < 0.05$).

McKenzie et al. then investigated if ICSI improved reproductive outcomes in men with 0% normal forms when compared to men with > 0% normal forms [36]. The investigators retrospectively analyzed 3 years of ICSI outcomes using Kruger's strict criteria, similar criteria to that used by Pisarsaka et al. [46]. Eight percent (45/545 cycles) of men in this time period had 0% normal forms. Fertilization rates and pregnancy rates were not statistically significantly different ($p > 0.05$) in those with 0% or > 0% normal forms [46]. Similarly, Hotaling et al. recently conducted a meta-analysis of all literature from 1986 to 2009 pertaining to the impact of isolated teratozoospermia on outcomes of ART. The authors found that isolated teratozoospermia was not associated with lower clinical pregnancy rates with IVF with or without ICSI [35].

The impact of sperm morphology on pregnancy outcome has also been investigated in relation to motile sperm organelle morphology examination (MSOME), a higher resolution microscopic technique. This method utilizes the Nomarski interference microscopy, which generates a total magnification of greater than × 6000, thereby providing a new morphological criterion: the presence of nuclear vacuoles [47]. With this more refined and structured manner of assessing sperm morphology, intracytoplasmic morphologically selected sperm injection (IMSI) was developed. It was initially thought that IMSI would be appropriate for couples with previously failed ICSI [48, 49].

Antinori et al. conducted a prospective randomized trial comparing IMSI to conventional ICSI, investigating the potential advantage of IMSI in the treatment of patients with severe oligoasthenoteratozoospermia regardless of prior ICSI outcomes [50]. In 446 couples, IMSI resulted in higher implantation rates (17.3% versus 11.3%, $p = 0.007$) and higher clinical pregnancy rates than conventional ICSI (39.2% versus 26.5%, $p = 0.004$). When couples were divided by the number of previously failed ICSI attempts (0, 1, or ≥ 2), pregnancy rate favored IMSI in every subgroup, but only reached statistical significance in the subgroup of couples who had failed ≥ 2 ICSI cycles ($p = 0.017$). There was no significant difference in miscarriage rate among the two cohorts. It is important to note that men selected for this study were not with isolated morphologic abnormalities, but also men with low concentration ($< 5 \times 10^6$/ml) and low motility (< 20% progressive motility). The authors concluded that IMSI was a better option than ICSI in cases of severely abnormal semen parameters, although not specifically abnormal morphology.

With the evolution of sperm morphology criteria, current studies have examined the utility of ICSI in cases specifically of teratozoospermia [51]. Using the WHO 5th edition criteria, Li et al. studied the predictive value of normal sperm morphology rate conventional IVF versus ICSI. Investigators retrospectively compared 4765 infertile couples being treated with either conventional IVF (3922 couples) versus ICSI (843 couples), where patients were grouped by normal sperm morphology rate ≥ 14%, 4–14%, and < 4% [52]. The authors found that in the conventional IVF cohort, fertilization rates decreased as normal sperm morphology rate decreased ($p < 0.05$). But in the ICSI cohort, fertilization rates did not correlate with normal sperm morphology rates ($p > 0.05$). Additionally, the miscarriage rate was significantly higher when normal sperm morphology rate was < 4% in the conventional IVF cohort ($p < 0.001$). In contrast, normal sperm morphology rate did not correlate with implantation, clinical pregnancy, live birth, or miscarriage rates in the ICSI cohort ($p > 0.05$). The authors concluded that ICSI should be the favored ART method for men with normal sperm morphology rate < 4% [52].

In contrast, van den Hoven et al. demonstrated poor prognostic value of sperm morphology as a predictor for pregnancy outcomes with respect to IVF versus ICSI [51]. Investigators analyzed the clinical significance of sperm morphology and pregnancy outcomes over a 25-year period. By analyzing data over such a long period of time, investigators sought to focus on the clinical impact of the evolving WHO morphologic criteria from the 1st through 5th WHO editions. The author then examined the relationship between sperm morphology and conventional IVF ($n = 2323$) versus ICSI ($n = 1353$). It is worthwhile to mention that in the Netherlands, due to the variability of acceptable normal sperm morphology, the decision to pursue conventional IVF versus ICSI has been based primarily on the total progressively motile sperm count in ejaculate, not the percent of morphologically normal sperm. The authors found that sperm morphology did not affect the odds of pregnancy via conventional IVF or ICSI ($p > 0.05$) [53].

In summary, the existing data on the impact of sperm morphology on IVF and ICSI outcomes is conflicting. While there are studies showing that morphology affects reproductive outcomes, more recent data show that fertilization and clinical pregnancies have been reported in couples even with normal sperm morphology of 0% [19]. More prospective data is needed in this area to define the true impact and the role for IVF and ICSI in affected couples.

Embryo Quality and Development

Maternal factors have been long known to contribute to embryo quality [53], yet the impact of male factors is still being determined [53, 54]. Parinaud et al. found that sperm with morphologic abnormalities of the post-acrosomal region and sperm with cytoplasmic droplets resulted in embryos of a lower quality [55].

Similarly, a 2018 study by Coban et al. found a relationship between sperm morphology and embryo aneuploidy. Donor oocytes were used to minimize the impact of the maternal factor on aneuploidy [56]. A total of 1165 embryos were divided by sperm morphology according to Kruger's strict criteria (score groups 1–5, where a higher score indicated better morphology). While fertilization rates improved with increasing morphology score, this was not statistically significant. However, mean incidence of aneuploidy was lower in group 5 compared to the other groups with lower morphology scores ($p < 0.003$). The true impact of sperm morphology on embryo quality and development is unclear based on the very little evidence in this area.

Morphology and Advanced Paternal Age

Sperm morphology has been shown to decline with advancing paternal age [57, 58]. A review of the impact of advancing paternal age and semen parameters and reproductive outcomes found that [57] there was an increase in the incidence of sperm head:width ratio (sperm head elongation) in men > 41 years old, which coincided with a lower rate of morphologically normal sperm (using Kruger's criteria) in men over age 40 [58]. Interestingly, in their aneuploidy studies, Coban et al. did not find a correlation between paternal age and total aneuploidy ($p = 0.202$), trisomy ($p = 0.290$), monosomy (0.079), and fertilization rates (0.848) [56].

Limitations in Measuring Sperm Morphology

The conflicting findings and lack of a clear consensus on the effect of sperm morphology on reproductive outcomes may be related to a variety of factors. There has been a lack of consensus on a universal method of classification [59]. One laboratory may use the strict Tygerberg criteria, defining teratozoospermia as normal morphology < 5%, while another laboratory may use the WHO 4th edition criteria, defining teratozoospermia as normal morphology < 15% [35]. In addition, the assessment of sperm morphology may be subject to sample errors. Only 200 sperm are assessed, a tiny proportion of the millions present in a given sample (which may also vary between days for a given individual) [10]. Individual lab staining techniques and preparation of smears may vary as well. The WHO 5th edition recommends Papanicolaou stain for the best morphological assessment. However, a given lab may use Shorr stain and Diff-Quik stain for faster, yet less detailed, results [60, 61].

Further complicating this issue, manual assessment of sperm morphology is visually subjective [59]. In a study assessing for inter-observer variability of 20 different laboratories' results of sperm morphology and sperm antibody levels, there was wide variation, even within one institution using the same assessment tool. Between labs, sperm morphology measurements have been shown to vary, and 40% of labs had a coefficient of variance/variation (CV) between 10 and 20%, and 3 labs had a CV > 20%, indicating wide inter-lab variability [62].

Similarly, after analyzing evaluations of three experts from different hospitals in their analyses of 5296 sperm samples from anonymous donors utilizing the WHO 5th edition, Wang et al. found the CV to be only 4.80%. Interestingly, there was a higher CV for recognition of head defects and cytoplasm defects than for midpiece and tail defects. Coefficients of agreement between any two of the three evaluators for overall sperm morphology were "moderate" [63]. Likewise, Eustache and Auger conducted an external quality assessment investigation. Sperm morphology was studied via evaluating inter-individual variability in the recognition of normal and abnormal sperm using high-resolution images of sperm projected from a video-equipped microscope. The overall coefficient of variation for the percentage of normal sperm was found to be 40% [59]. Because of these limitations, the clinical impact of sperm morphology continues to be heterogeneous [50].

Conclusions

Sperm morphology is commonly assessed when a male presents for fertility evaluation, but the clinical utility of this parameter is currently under scrutiny. With the various renditions of the WHO manual for semen analysis testing, the barometer "normal" sperm has gotten progressively smaller. While historical data showed a predictive value for sperm morphology in reproductive outcomes, current data do not demonstrate this. In fact, the increase in strictness seems to have decreased the utility for sperm morphology in couples using ART [21••]. Currently, sperm morphology has a poor clinical impact on ART and natural pregnancy outcomes. Reinforcing this, the most recent data show that men with very poor sperm morphology (or no normal appearing sperm) seem to perform as well as men with normal sperm morphology. As men with complete absence of normal sperm morphology exhibit high rates of spontaneous and assisted pregnancy [20••]. While these data are complicated by heterogeneity in the preparation and reading of smears, variable classification systems, inter-observer variation, and the subjective nature of morphological assessment, it is clear that sperm form and

function are two distinct properties. The current literature shows that abnormal sperm morphology is no longer predictive of poorer reproductive outcomes across all ART types [21••, 35]. Because of this data, the American Society for Reproductive Medicine now states that there is no consensus on the influence of abnormal sperm morphology in the selection of a particular ART method [64].

Compliance with Ethical Standards

Conflict of Interest Rachel B. Danis and Mary K. Samplaski each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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