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REVIEW

Review Exploring Sperm Morphological: World Health Organization vs. Kruger Strict Criteria

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Abstract

Sperm morphology, the study of the size, shape, and appearance of sperm cells, is vital in evaluating male fertility. This assessment has evolved through decades of research, beginning in the 1930s with foundational classification systems by Cary and Moench.

A significant advancement came in the late 1980s with the Kruger strict criteria, which standardized sperm morphology evaluation using high-magnification microscopy and advanced staining, supported by the WHO.

This standardization is crucial in diagnosing male infertility and optimizing treatment strategies. In assisted reproduction technologies like IVF and ICSI, normal sperm morphology is essential for predicting fertilization potential and successful pregnancy outcomes.

The review article aims to explore the historical evolution, scientific advancements, and standardization efforts in sperm morphology assessment, focusing on its impact on diagnostic accuracy and treatment efficacy in male infertility and ART.

Keywords: Assisted reproduction technologies, Sperm morphology, World Health Organization criteria, Kruger strict criteria, Infertility

Introduction

Sperm morphology refers to the size and shape of sperm cells as observed under a microscope; it is an important aspect of semen analysis in assessing male fertility (Menkveld et al., 2011). Normal sperm morphology typically involves evaluating the percentage of sperm with normal shape and structure, as abnormalities in morphology can affect sperm motility and ability to fertilize an egg (Chemes & Rawe, 2003). A sperm with good morphology is typically oval-shaped with a smooth, regular outline, a well-defined head, mid-piece, and tail (Franken & Kruger, 2004).

Sperm morphology assessment is a critical component of male fertility evaluation, providing insights into the structure and form of sperm cells, which are essential for successful fertilization (Garcia-Vazquez et al., 2016). The assessment typically in the labs focuses on identifying the percentage of sperm that exhibit normal morphology, which can significantly influence reproductive outcomes (Khatun et al., 2018).

Two predominant methodologies guide the evaluation of sperm morphology, which are World Health Organization criteria (WHO) and the Kruger Strict Criteria (KSC), the WHO criteria provide a comprehensive framework for assessing sperm morphology, including detailed guidelines on the classification of sperm head, mid-piece, and tail defects (Menkveld, 2010).

According to the latest WHO guidelines, normal sperm morphology is defined by specific parameters for the head, mid-piece, and tail (Menkveld et al., 2011). Head: The sperm head should be smooth, oval shape, with a well-defined Acrosome covering 40–70% of the head area. The length should be 4–5 μm, and the width should be 2.5–3.5 μm (Cengiz, 2023). Mid-piece: The mid-piece should be slender, approximately the same length as the head, and well defined, with a regular appearance (Ghanbarikeshteli, 2024). Tail: The tail should be uniform, uncoiled, and approximately 45 μm in length (Nassir et al., 2024). According to the WHO, a semen sample is considered within normal range if at least 4% sperm forms in 1 mL of the semen sample observed have

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normal morphology (Menkveld et al., 1990). This threshold is based on the understanding that even a small proportion of normally shaped sperm can be sufficient for natural conception, especially when the overall sperm count and motility are within normal ranges (Dias et al., 2019).

In contrast, the Kruger Strict Criteria offer a more stringent assessment, focusing primarily on the sperm head's shape and size, which are crucial for its ability to penetrate and fertilize the egg (Oehninger & Kruger, 2021). This method sets a higher threshold for what is considered normal, thereby providing a more selective and predictive measure of fertilizing potential (Silverberg & Turner, 2017a). The Kruger Strict Criteria offer a more stringent and detailed method for assessing sperm morphology, placing greater emphasis on the sperm head's dimensions and shape, which are critical for successful fertilization (Nikolettos et al., 1999). Under this criteria, Head: The sperm head must be precisely oval with a smooth contour, and deviations in size or shape are more stringently categorized as abnormalities (Barroso et al., 1999). The Acrosome should occupy 40–70% of the head area, similar to WHO standards, but with stricter adherence to these measurements (Milachich & Dyulgerova-Nikolova, 2020a). Mid-piece and Tail: The mid-piece should be regular and axially attached to the head, while the tail should be straight and uniform (Edition, 2010). Any deviations from these norms are considered defects (Menkveld, 2007). The Kruger method sets a higher bar for normal morphology, often requiring at least 14% of sperm to meet these strict criteria to be considered within the normal range (Maree et al., 2010). This more rigorous approach is especially valuable in assisted reproductive technologies (ART), where the precise morphology of sperm is critical for procedures like in vitro fertilization (IVF) and Intra-Cytoplasmic Sperm Injection (ICSI) (Marzano et al., 2020).

The use of both WHO and Kruger Strict Criteria in clinical practice allows for a nuanced understanding of male fertility potential. While the WHO criteria provide a broader and more inclusive assessment, the Kruger Strict Criteria offer a more selective measure, which can be particularly useful in predicting the outcomes of ART procedures (Sharma et al., 2010).

Both standards provide frameworks for analyzing sperm morphology, but they differ in their methodologies, diagnostic significance, and clinical implications. This comprehensive comparison aims to highlight these differences and offer an analysis of their respective benefits in clinical practice in providing a solution to the problem of male infertility.

Material and methods

An extensive and systematic review of relevant literature was conducted using multiple electronic scientific databases. These databases included Scopus, Science Direct, Springer Link, PubMed, Google Scholar, and Cochrane Systematic Reviews. The comprehensive search targeted published articles and books during the twentieth century and in the first three decades of the twenty-first century. Each database was thoroughly examined to ensure a wide-ranging collection of pertinent studies and publications that contribute to the research topic. The search strategy was designed to identify and compile a robust body of literature to support the study's objectives.

History of criteria development in evaluating sperm morphology

Early contributions to the field of sperm morphology assessment emerged in the early 1930s, driven by pioneers such as Cary and Moench, Holt, Williams, and Hammen, among others (Leikkola, 1955). These researchers laid foundational groundwork that continues to shape modern andrology and reproductive medicine. Cary and Moench proposed early classification systems aimed at categorizing sperm based on observable structural characteristics, highlighting the need for standardized criteria to distinguish normal from abnormal spermatozoa (Perelman et al., 2014). Concurrently, Holt advanced staining techniques to enhance visibility and differentiation of sperm structures, thereby improving the accuracy of morphological analysis in early andrology laboratories (Czubaszek et al., 2019).

In 1937, Williams expanded upon previous classification systems, refining criteria that defined normal and abnormal sperm morphology more precisely, his contributions underscored the importance of comprehensive morphological assessment in diagnosing male infertility (Menkveld, 2010). Building on these advancements, Hammen's work in 1944 further refined classification systems, identifying specific morphological features linked to fertility potential and emphasizing the clinical significance of sperm morphology assessment (Mann & Lutwak-Mann, 2012).

During the late 1940s and early 1950s, MacLeod introduced a systematic approach categorizing abnormal sperm head forms into distinct classes, establishing baseline criteria for assessing male fertility potential (Ombelet et al., 1995). However, his initial focus on head morphology did not encompass abnormalities in other sperm regions, which were recognized as significant but initially not included in his classification system (Auger et al., 2016).

A significant milestone came in the late 1970s with Dr. Guido David's classification system, which standardized sperm morphology assessment by introducing clear terminology and strict criteria for categorizing abnormalities across the entire spermatozoon—head, neck, mid-piece, and tail (Chang et al., 2017). This system revolutionized male fertility assessment, linking specific abnormalities to reduced fertilization rates and guiding treatment decisions in assisted reproduction.

In 1971, Eliasson advocated for a holistic approach to sperm morphology assessment, emphasizing evaluation of the entire spermatozoon—head, mid-piece, and tail—which reshaped clinical practices and enhanced diagnostic accuracy worldwide (Carson & Kallen, 2021).

The late 1980s saw the development of the Kruger strict criteria, which set standardized guidelines for assessing sperm morphology, defining normal characteristics and employing rigorous methodologies like high-magnification microscopy and standardized staining techniques (Menkveld et al., 2001). Widely adopted in clinical practice, these criteria inform treatment decisions and predict fertilization outcomes in assisted reproduction. The World Health Organization (WHO) has been pivotal in advancing and standardizing criteria for assessing morphologically normal spermatozoa since the publication of their seminal manual in 1980 (Menkveld, 2010).

The World Health Organization criteria

The WHO criteria for sperm morphology represent a pivotal standard in the assessment of male fertility (Nallella et al., 2006). Since the publication of their seminal "WHO Laboratory Manual for the Examination and Processing of Human Semen" in 1980, the WHO has played a crucial role in establishing guidelines that define normal sperm morphology based on specific characteristics of the head, neck, midpiece, and tail (Gatimel et al., 2017). According to these criteria, an oval shape with smooth contours, measuring 4.0–5.5 μm in length and 2.5–3.5 μm in width, defines normal sperm head morphology (Hoogendijk, 2007). The acrosome should cover 40–70% of the head's surface and appear well defined and smooth, while the nucleus should be uniformly stained and occupy most of the head without vacuoles exceeding 20% of its area (Silverberg & Turner, 2017b). Abnormalities encompass various deviations such as tapered, amorphous, round (globozoospermia), large, small, vacuolated, double, and pinheads (Mortimer, 1994). Furthermore, normal sperm mid-piece morphology, as per WHO criteria, features a slender, straight, cylindrical shape that is about 1.5 times the length of the head (Patel, 2023). It should align axially with the head and tail, maintaining a streamlined structure essential for efficient motility. The mid-piece houses mitochondria arranged in a spiral around the axoneme, crucial for generating energy to power sperm movement (Cummins, 2009). As well as, abnormal sperm mid-piece morphology, defined by WHO criteria, encompasses deviations that can impair sperm motility and functionality (Sunanda et al., 2018). These include bent or angulated mid-pieces, excessively thick or thin mid-pieces, irregular contours, and the presence of cytoplasmic droplets, absence, or duplication of the mid-piece (Cooper, 2005). Each abnormality can affect the sperm's ability to move effectively, hindering fertility potential (Chemes & Rawe, 2003). According to WHO standards for sperm morphology, normal sperm tail morphology is characterized by a thin, straight structure approximately 45–50 μm long, with consistent width, proper alignment with the head and mid-piece, flexibility, and regular whip-like movements for progressive motility (Menkveld, 2010). Abnormalities in tail morphology, such as coiled or curled tails, bent or angulated tails, short or double tails, thickened tails, irregular contours, fragile tails, or complete absence of tails, can significantly influence sperm motility and overall functionality (Siddique et al., 2011). In Fig. 1, view the World Health Organization's 2021 Guide to Diff-Quick stain, identifying normal and abnormal sperm form (World Health Organization, 2021) (see Fig. 2).

Threshold for normal morphology according to WHO criteria

The World Health Organization (WHO) criteria for sperm morphology set a crucial standard for assessing male fertility potential by defining specific parameters for the head, mid-piece, and tail (Milachich & Dyulgerova-Nikolova, 2020b). These criteria ensure uniformity and objectivity in semen analysis across clinical and research environments (Agarwal et al., 2022a). Normal sperm morphology, as per WHO guidelines updated in 2021, requires that at least 4% of spermatozoa in a sample exhibit typical structural characteristics such as oval heads with well-defined acrosomes, straight mid-pieces containing densely packed mitochondria, and tails that are uniformly wide and flexible for effective motility

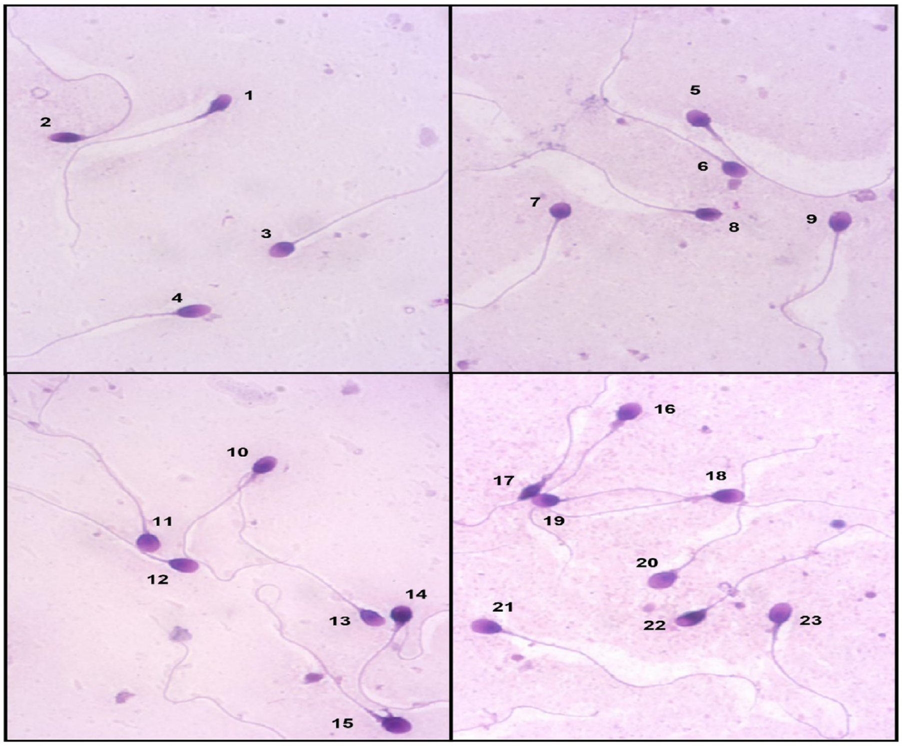


Fig. 1. ***0*** *Diff-Quick plate (****1;****H = abnormal, M = normal, T = normal.* ***2;****H = abnormal, M = thick, T = normal.* ***3;****H = normal, M = normal, T = normal.* ***4;****H = abnormal (not oval),M = normal, T = normal.* ***5;****H = abnormal (amorphus),M = thick, T = normal.* ***6;****H = normal, M = normal, T = normal.* ***7;****H = abnormal (not oval),M = normal, T = normal.* ***8;****H = abnormal, M = normal, T = normal.* ***9;****H = normal, M = normal, T = normal.* ***10;****H = abnormal (not oval),M = thick, T = normal.* ***11;****H = normal, M = normal, T = normal.* ***12;****H = normal, M = normal, T = normal.* ***13;****H = abnormal (tapered),M = normal, T = normal.* ***14;*** *H = abnormal (small),M = thick, T = normal.* ***15;****H = normal, M = thick, T = normal.* ***16;****H = abnormal (amorphus), M = thick, T = normal.* ***17;****H = abnormal (tapered), M = thick, T = normal.* ***18;****H = normal, M = asymmetrical, T = normal.* ***19;****H = abnormal (not oval),M = thick, T = normal.* ***20;****H = normal, M = normal, T = normal.* ***21;****H = normal, M = normal, T = normal.* ***22;****H = abnormal, M = normal, T = normal.* ***23;****H = abnormal (not oval), M = asymmetrical, T = normal).*
H= Head, M = Mid-piece, T = Tail.

(Minhas et al., 2021). Adherence to these criteria plays a pivotal role in diagnosing male infertility (Dohle et al., 2004). Deviations from the established morphology standards, such as abnormalities in head, mid-piece, or tail morphology—such as tapered heads, coiled tails, or the presence of cytoplasmic droplets—can significantly impair sperm motility and function, thereby contributing to fertility challenges (Haidl & Schuppe, 2006). Table 1; which displaying the typical values from 1980 to 2021 (Esteves et al., 2012).

The Kruger Strict Criteria for sperm morphology

The KSC represent a meticulously detailed framework for evaluating sperm morphology, emphasizing precise assessment of the head, mid-piece, and tail characteristics (Popova, 2021). Developed by Dr. Thinus Kruger, this method is renowned for its stringent parameters, crucial for predicting success in assisted reproductive technologies (ART) such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (Kruger et al., 1987). Head sperm morphology assessment under the Kruger Strict Criteria demands strict adherence to specific standards: the head must be smooth, oval-shaped with a well-defined acrosome covering 40–70% of its surface, and measure 4–5 μm in length and 2.5–3.5 μm in width (van Rensburg, 1998). Any deviations, such as tapered, pyriform, round, or irregularly sized heads, are classified as abnormalities, ensuring only sperm with optimal fertilization potential are deemed normal. The evaluation of the mid-piece focuses on its slender, symmetrical shape aligned with the head, devoid of cytoplasmic droplets larger than one-third of the head size—a sign of efficient energy production for motility (Immegart, 1996). Abnormal mid-piece structures, such as thickened or bent shapes, are noted for their potential to affect sperm motility (De Boer et al., 2015). Tail morphology, critical for sperm movement, is meticulously evaluated for length, straightness, and absence of abnormalities like coils or multiple tails (Ayad, 2018). The Kruger Strict

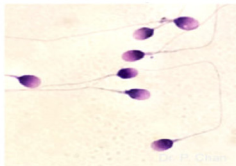
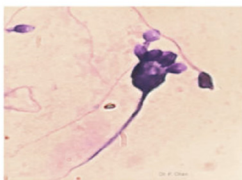
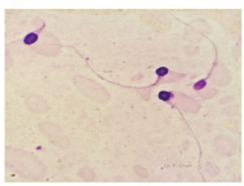
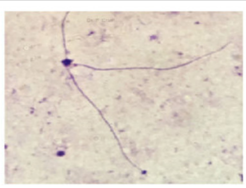
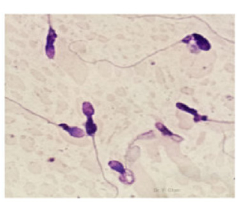
Pictures	Comments
	Normal sperm have an oval-shaped head, an unbroken mid-piece, and a straight, uncoiled tail, which allows them to swim properly, and they carry a healthy genetic load.
	Macrocephaly in sperm refers to a disorder in which the sperm head is excessively big, which frequently results in additional chromosomes and difficulty fertilizing the egg.
	Microcephaly in sperm refers to a condition in which the head is smaller than normal, also known as small-head sperm, which is required for the sperm to reach the egg, or it may entail a loss in genetic material.
	Pinhead sperm, a subtype of small-head sperm, is characterized by a pin-like head and low to no paternal DNA content. Pinhead sperm may indicate a diabetic state.
	Tapered head sperm have elongated, "cigar-shaped" heads that can indicate the presence of a varicocele or frequent exposure of the scrotum to high temperatures, such as in daily sauna use. Additionally, sperm frequently exhibit abnormal chromatin or packaging of paternal DNA, potentially leading to aneuploidy—a condition characterized by an abnormal number of chromosomes in the sperm.

Fig. 2. Displays the sperm shapes based on Kruger's classification (Sperm morphology, 2018).

Criteria establish a stringent standard for normal sperm morphology, stipulating that a semen sample be considered within the normal range if at least 14% of the observed sperm exhibit typical structural characteristics (Brooks, 2005). This classification indicates a high probability of fertility; samples with 4–14% normal morphology suggest a slight decrease in fertility potential, while samples showing 0–3% normal morphology indicate significantly impaired fertility (Enciso et al., 2011). This stringent threshold ensures that sperm selected for ART procedures meet the highest standards for potential fertilization success (Said & Land, 2011). In clinical practice, adherence to the Kruger Strict Criteria is particularly vital in ART contexts, where sperm quality directly influences the outcomes of fertility treatments like IVF and ICSI (Barrera et al., 2022) (see Fig. 2 Sperm Morphology, 2018).

WHO and Kruger criteria in sperm morphology for ART

The role of sperm morphology with assisted reproductive technology is pivotal to the success of fertility treatments such as in vitro fertilization and Intra-Cytoplasmic Sperm Injection (Gatimel et al., 2017). Sperm morphology, defined by the size, shape, and structural integrity of the sperm, directly influences its ability to fertilize an egg and ultimately impact pregnancy outcomes (Oehninger & Kruger,

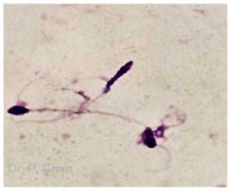
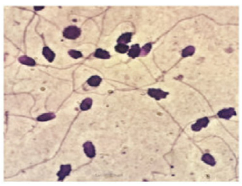
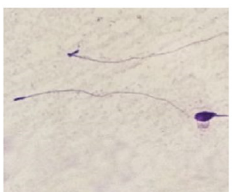
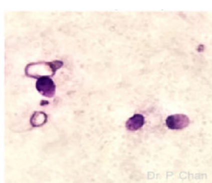
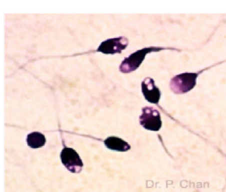
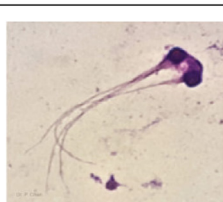
	Thin head sperm are an extreme variety of tapered head sperm, with a thin and elongated head shape, they are infrequently seen and may signal concerns such as DNA fragmentation, varicocele, or anomalies in head development.
	Globozoospermia, defined by round-headed sperm, suggests problems such as the lack of the acrosome or the absence of inner components required to activate the egg during fertilization. Decondensing head sperm is a variation of this disorder in which the sperm nucleus prematurely breaks down, allowing the DNA material to unravel and fill the whole sperm head.
	Headless sperm, also known as acephalic sperm or decapitated sperm syndrome, lack a sperm head and thus do not contain genetic material or chromosomes, while they may appear to be pinhead sperm at first glance, closer examination reveals that they lack even a small head, resembling a loose string.
	Acaudate sperm, or sperm without a tail, are often seen in necrosis, a state in which an organ has experienced extensive cell death.
	When two or more big, cyst-like bubbles or several microscopic vacuoles are evident under high magnification microscopy within the sperm head, they are referred to as nuclear vacuoles in sperm.
	Sperm with many heads or tails are examples of anomalies that might be seen. Duplicate sperm is the word for sperm that have two heads; this condition is linked to exposure to heavy metals like cesium, smoking, and increased levels of prolactin hormone in men.

Fig. 2. (Continued).

2021). In ART procedures, selecting sperm with normal morphology, as determined by rigorous criteria like WHO and the Kruger Strict Criteria, is crucial (Menkveld et al., 1990). Normal sperm morphology ensures that the spermatozoon possesses the structural integrity necessary for penetrating and fertilizing the egg (Ombelet et al., 1995). Sperm with abnormal morphology, such as irregularly shaped heads, bent mid-pieces, or coiled tails, may have reduced motility or impaired ability to bind to the egg, thereby affecting fertilization rates (Chemes & Rawe, 2003). Clinicians and embryologists employ stringent criteria, such as WHO and the Kruger Strict Criteria, to assess sperm morphology

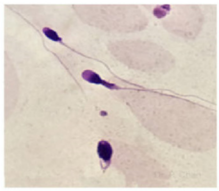
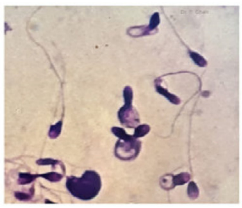
	Large swellings in the midpiece or neck of sperm may be a sign of malfunctioning mitochondria, which are essential for the sperm cell's ability to produce energy. Additionally, it could point to anomalies in the centrioles, which direct chromosomal arrangement and movement inside the sperm neck.
	Bacterial presence or exposure to inappropriate seminal fluid conditions are commonly linked to coiled-tail sperm. These sperm have broken tails, which makes it difficult for them to swim properly. There is a connection between higher levels of heavy smoking and coiled-tail sperm.
	Sperm cells with short tails, also known as stump tail or Dysplasia of Fibrous Sheath (DFS) sperm, frequently have minimal or no motility. This tail abnormality develops during late spermiogenesis in the testis. Individuals with DFS may also have persistent respiratory disorders such immotile cilia syndrome. DFS is an autosomal recessive genetic illness caused by faulty genes that encode proteins such as sperm neck centrin. It is connected with an increased risk of sperm aneuploidies, which are chromosomal abnormalities.

Fig. 2. (Continued).

Table 1. The reference values for sperm as per publications from the World Health Organization, spanning from the first to the sixth edition.

Parameters according to WHO	1st edition	2nd edition	3rd edition	4th edition	5th edition	6th edition
Volume (mL)	Not detect	≥2.0	≥2.0	≥2.0	≥1.5	≥1.5
Concentration (million/mL)	20–200	≥20	≥20	≥20	≥15	≥15
Total sperm motile (%)	≥60	≥50	≥50	≥50	≥40	≥39
Progressive motility (%)	≥25 (grad A)	≥25 (grad A)	≥25 (grad A)	≥25 (grad A)	≥32 (grad A + B)	≥32 (grad A + B)
Vitality (%)	Not detect	≥50	≥75	≥75	≥58	≥42
Normal form (%)	≥80.5	≥50	≥30	≥14	≥4	≥4

accurately (Kruger & Coetzee, 1999). These criteria help identify sperm with the highest potential for successful fertilization, thereby enhancing the chances of a successful ART cycle (Foxcroft et al., 2008). Techniques like sperm selection based on morphology using advanced microscopy technologies enable precise identification and isolation of sperm with optimal morphology for use in ART procedures (Dai et al., 2021). The success of ART procedures heavily relies on the quality of sperm morphology (Di Santo et al., 2012). Higher percentages of sperm with normal morphology correlate with improved fertilization rates, embryo development, and ultimately, higher chances of achieving a successful pregnancy. Conversely, abnormalities in sperm morphology can lead to lower success rates or even failure of ART cycles (De Vos et al., 2003). Sperm morphology can be a preliminary filter for selecting sperm with potentially better DNA integrity for ICSI (Parmegiani et al., 2014), While normal morphology, according to strict criteria like Kruger's, often suggests better DNA quality, even these sperm may still have DNA fragmentation (Oehninger & Kruger, 2021). Abnormal morphology, especially with head defects, is usually linked to higher DNA fragmentation, though not all abnormalities affect DNA (Cassuto et al., 2012), Thus, combining morphology-based selection with advanced techniques like Intracytoplasmic Morphologically Selected Sperm Injection (IMSI), Physiological Intracytoplasmic Sperm Injection (PICSI), or direct DNA damage assays, such as the Halo test, improves the chances of choosing high-quality sperm for ICSI (Pic et al., 2020).

The WHO criteria are less stringent, allowing a broader range of normal sperm morphology, which can miss subtle chromosomal abnormalities (Tournaye et al., 2017), Head defects like large or double heads are linked to chromosomal aneuploidies, while tail defects may indicate DNA fragmentation, yet these may go undetected under WHO's standards (Dariš et al., 2010) but, Kruger's strict criteria demand nearly perfect morphology, closely correlating with better DNA and chromosomal integrity (Henkel et al., 2010). Abnormalities such as pin, pyriform, tapered, vacuoles or asymmetrical heads, even minor ones, are associated with chromosomal defects, making Kruger-selected sperm less likely to carry genetic issues than those meeting WHO's broader criteria (Mangiarini et al., 2013).

ICSI outcomes—such as fertilization, cleavage rates, and embryo quality—vary depending on the criteria used. The WHO's broader criteria may yield acceptable fertilization rates but can include sperm with minor defects, potentially leading to lower cleavage rates and reduced embryo quality and leading to a decrease in success rate (Lundin et al., 2001), Kruger's strict criteria, however, select sperm with nearly perfect morphology, often resulting in higher fertilization rates, improved cleavage, and better embryo quality (Grow et al., 1994). This selection of morphologically ideal sperm aligns with fewer chromosomal issues, increasing the chances of high-quality embryos and better pregnancy outcomes (Li et al., 2014).

Basic treatment techniques for abnormal sperm shape

Treatment strategies for abnormal sperm morphology vary depending on the underlying causes and the specific needs of individuals or couples.

1. Lifestyle Modifications: Adopting a healthy lifestyle can often enhance sperm morphology, which includes maintaining a balanced diet, regular exercise, reducing alcohol consumption, quitting smoking, and managing stress levels (Agarwal & Durairajanayagam, 2015).
2. Medical Interventions: Addressing hormonal imbalances through medications that regulate hormone levels can be beneficial in treating abnormal sperm morphology (Brezina et al., 2012).
3. Surgical Procedures: Correcting structural issues such as varicoceles (enlarged veins in the scrotum) through surgery may improve sperm morphology (Morini et al., 2021).
4. Assisted Reproductive Techniques: Procedures like intrauterine insemination (IUI) or in vitro fertilization (IVF) can facilitate conception by directly placing sperm into the female reproductive tract, bypassing barriers posed by abnormal sperm morphology (Leung et al., 2022).
5. Nutritional Supplements: Certain supplements, such as antioxidants (e.g., vitamin E, vitamin C), coenzyme Q10, and zinc, may be recommended to enhance sperm morphology and overall sperm health (Walczak– et al., 2013).
6. Environmental Management: Minimizing exposure to environmental toxins and heat sources (like hot tubs or saunas) that can adversely affect sperm health is crucial for improving sperm morphology (Durairajanayagam et al., 2014).

Evaluation techniques for sperm morphology

In the field of sperm morphology assessment, various staining techniques play crucial roles in enhancing the visibility and detailed analysis of sperm cells under the microscope. These stains serve to highlight specific structures and characteristics of spermatozoa, aiding in the diagnosis of male infertility and guiding treatment strategies in assisted reproduction (Lingappa et al., 2015).

1. Diff-Quik Stain: It is a rapid staining technique that provides quick differentiation of sperm morphology, which is commonly used in clinical settings for basic assessments, allowing for the visualization of sperm head and tail structures with ease (Natali et al., 2013).
2. Papanicolaou (PAP) Stain: PAP stain is known for its ability to differentiate various components of cells based on their affinity for acidic or basic dyes. In sperm morphology, PAP stain highlights nuclear morphology and chromatin integrity, aiding in the assessment of sperm DNA fragmentation and maturity (Veuthey et al., 2014).
3. Aniline Blue Stain: It is particularly useful in highlighting sperm chromatin condensation. It stains immature sperm with high chromatin density, aiding in the identification of sperm with potential DNA damage, which can affect fertility outcomes (Dutta et al., 2021).
4. Giemsa Stain: It is widely used in cytology and histology for its ability to stain acidic and basic components of cells differently, Giemsa stain in sperm morphology provides contrast that helps identify nuclear abnormalities and assess sperm viability (Zafar et al., 2020).
5. Hematoxylin-Eosin (H&E) Stain: H&E stain is a routine histological stain that colors nuclei blue (with hematoxylin), cytoplasm, and other structures pink or red (with eosin), that is used in sperm morphology to differentiate cellular components and assess overall tissue structure

in testicular biopsies or sperm smears (Chan, 2014).

6. Methylene Blue-Eosin (MBE) Stain: this stain is a differential stain used to highlight specific cellular components based on their chemical properties. In sperm morphology, MBE stain helps in distinguishing between live and dead sperm cells, providing valuable information about sperm viability (Dibal et al., 2022).
7. Toluidine Blue Stain: It is used to assess sperm morphology by staining specific structures such as sperm heads and tails. It highlights cellular features and abnormalities, aiding in the detailed examination of sperm morphology and function (Alves et al., 2018).
8. Spermac staining: Also known as, Sperm Chromatin Dispersion (SCD) test or Sperm Chromatin Structure Assay (SCSA) is a diagnostic technique used in the evaluation of male fertility, fragmented or damaged DNA fluoresces red, indicating poor chromatin integrity and suggesting potential fertility issues (Oettlé, 1986).

Discussion

During a period of two decades ago, the assessment of sperm morphology has experienced substantial development. Beginning in the early 1930s, groundbreaking work by scientists such as Cary, Moench, Holt, Williams, and Hammen established the fundamental principles of contemporary andrology and reproductive medicine (Menkveld et al., 2011). These early researchers introduced classification systems aimed at categorizing sperm based on observable structural characteristics, highlighting the necessity for standardized criteria to distinguish normal from abnormal spermatozoa (Chang et al., 2017). Cary and Moench's early classification systems set the stage for subsequent refinements in morphological assessment, paving the way for more precise diagnostic tools (Menkveld et al., 2011).

In the late 1980s, the development of the Kruger strict criteria marked a significant milestone by establishing standardized guidelines for assessing sperm morphology (Kruger & Botha, 2007). Utilizing high-magnification microscopy and advanced staining techniques, these criteria rigorously define normal sperm characteristics (Maettner et al., 2014). Widely adopted in clinical practice, they inform treatment decisions and predict fertilization outcomes in assisted reproduction technology (Hanassab et al., 2024), similarly, in World Health Organization has played a pivotal role since 1980 in advancing and standardizing criteria for assessing morphologically normal spermatozoa (Gatimel et al., 2017). The WHO criteria, periodically updated, define normal sperm morphology based on specific parameters of the head, mid-piece, and tail, ensuring consistency and objectivity in semen analysis across clinical and research environments (Agarwal et al., 2022b). This standardization supports accurate diagnosis and tailored treatment strategies for male infertility, thereby improving patient outcomes (Pierik et al., 2000). Then, assessing the percentage of abnormalities like thin heads, amorphous heads, or bent and asymmetrical necks has limited clinical value, as these features are often physiological traits with poorly understood pathophysiology.

The pivotal role of sperm morphology in ART procedures such as in vitro fertilization and intracytoplasmic sperm injection cannot be overstated (Said & Land, 2011). Sperm morphology, encompassing size, shape, and structural integrity, directly influences fertilization potential and influences pregnancy rate outcomes (Chemes & Rawe, 2003). Selection of sperm with normal morphology, as assessed by rigorous criteria same as the Kruger Strict Criteria, is crucial in ART (Oehninger & Kruger, 2021). Normal sperm morphology ensures the structural integrity necessary for spermatozoa to penetrate and fertilize the egg. Conversely, sperm with abnormal morphology, characterized by irregularities in head shape, mid-piece bending, or tail coiling, may exhibit reduced motility or impaired ability to bind to the egg, thus affecting fertilization rates (Graham, 2001). Clinicians and embryologists rely on stringent criteria to accurately assess sperm morphology, enhancing the likelihood of successful ART cycles (Kohn et al., 2018). So that, some studies have found correlations between the percentage of normal sperm forms and functional abnormalities, as well as the ability to conceive naturally or, in some cases, the success of intrauterine insemination (IUI) and conventional IVF.

Advanced microscopy technologies enable precise identification and isolation of sperm with optimal morphology, enhancing the success rates of ART procedures (Dai et al., 2021). Higher percentages of sperm with normal morphology correlate with improved fertilization rates, embryo development, and increased chances of achieving successful pregnancies (De Vos et al., 2003), conversely; abnormalities in sperm morphology can lead to lower success rates or even failure of ART cycles, underscoring the critical role of precise morphology assessment in clinical outcomes (Villani et al., 2022).

In the realm of sperm morphology assessment, various staining techniques play essential roles by enhancing the visibility and detailed analysis of sperm cells under the microscope (Hekmatdoost et al., 2009). Techniques such as Diff-Quik, Papanicolaou (PAP), Aniline Blue, Giemsa stain, Hematoxylin-Eosin (H&E), Methylene Blue-Eosin (MBE), and Toluidine Blue stains highlight specific sperm structures and characteristics (Carson, 2015), which these stains aid in diagnosing male infertility by providing insights into sperm chromatin integrity, viability, and morphological abnormalities, thereby guiding appropriate treatment strategies in assisted reproduction (Agarwal & Sharma, 2023).

In summary, the development of sperm morphology evaluation over time has been characterized by a progressive path that has been aided by groundbreaking discoveries and scientific breakthroughs. In the area of male infertility and ART, standardized criteria and cutting-edge laboratory techniques continue to propel advancements in diagnostic precision, therapeutic effectiveness, and patient outcomes.

Conclusion

The study of sperm morphology has advanced significantly over the decades, driven by pioneering research and continuous technological innovations. Beginning with foundational work in the early 1930s by scientists like Cary and Moench, classification systems were introduced to categorize sperm based on observable structural traits, highlighting the need for standardized assessment criteria.

The establishment of the Kruger strict criteria in the late 1980s marked a pivotal milestone, standardizing guidelines for evaluating sperm morphology using advanced microscopy and staining techniques. These criteria, supported by the World Health Organization, have ensured consistency in semen analysis across clinical and research environments, crucial for diagnosing male infertility and optimizing treatment strategies.

In assisted reproduction technologies such as IVF and ICSI, sperm morphology plays a decisive role in determining fertilization success and influencing pregnancy outcomes. The selection of sperm with normal morphology is essential for achieving successful ART cycles, as abnormalities can diminish success rates.

Author contribution

The author strives to provide meaningful contributions to the research topic.

Ethics approval and consent to participate

Approval was obtained with no requirements.

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Conflict of interest

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