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The Effect of Environmental, Lifestyle, and Intrinsic Factors on Sperm Morphology in Assisted Reproduction

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Abstract
Sperm morphology as part of a complete semen analysis becomes more and more significant from a clinical point of view for infertility and perhaps men's health. The current manuscript reviews the effect of some environmental and lifestyle factors on sperm morphology. A new look into an old challenge is delivered based on contemporary scientific and clinical evidence. The impact of lifestyle, age of the male, heat stress, and other factors on sperm morphology, DNA fragmentation index (DFI), and, consequently, assisted reproductive techniques (ARTs) outcome is discussed. Sperm morphology is probably the most relevant parameter of the traditional semen evaluation and can be used as a valid biomarker assessment of male fertility potential in in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) programs.
Keywords: Sperm morphology, Assisted reproductive techniques, Sperm DNA fragmentation

Introduction

The clinical definition of infertility is a lack of pregnancy after 12 consecutive months of unprotected sexual intercourse. The incidence of infertility is 10–15% globally, and this has risen in recent years (1). Approximately 50% of infertility cases involve male factors, among which 20% are cases of pure "male factor" infertility, and approximately 30% are cases of combined female and male factor infertility (2).

Several studies revealed a wide variation in the estimation of the occurrence of male infertility (from 5% to 35%), thus showing fundamental differences between populations in terms of the following factors: quality of primary health care, environment, occupation, exposure to toxicants responsible for infertility, age, obesity, climate conditions, educational status, occasional use of or constant exposure to drugs, and genetic and epigenetic factors (3).

Currently, evaluation and diagnosis of male infertility mainly rely on traditional semen analysis, including the spermatozoa's volume, concentration, vitality, and morphology (4).

In men, the transformation of spermatids during spermiogenesis is the 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, the restructuring 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 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 upper endocervical canal's mucus have helped define a normal-shaped spermatozoon (5).

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 (6) 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.

Although the association between sperm morphology and assisted reproduction outcome (ART) is unknown, recent studies documented intriguing findings in male reproduction that merit further investigation.

Therefore, this review aimed to determine the effect of various environmental and lifestyle factors on sperm morphology on one hand and to find out the capability of spermatozoa to fertilize the oocytes on the other.

Evolution of the WHO "Semen" Processing Manual

The spermatozoon is a highly differentiated and polarized cell with two main structures: the head, containing a haploid nucleus, and the acrosome, containing exocytosis granule, and the flagellum, which generates energy (via

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mitochondria) and propels the cell; the neck connects both structures. The sperm aims to fertilize the oocyte and activate embryonic development. Despite this common bauplane and function, there is an enormous diversity in the structure and performance of sperm cells (7).

Data on semen quality collected systematically from reports published worldwide indicate that spermatozoa density has declined appreciably between 1938 and 1990 (8). In the 1940s, the consensus was that a volume of less than 1–1.5 mL after an ejaculatory pause of a couple of days or more is abnormal (9)

However, a semen volume of 1.4 mL has been in the sixth percentile and is considered a normal reference according to WHO 6th Edition 2021. The lower reference value for a "normal" sperm count has also changed from 60×10^6/mL in the 1940s (9) to the present value of 16 $\times 10^6$/mL (10).

The Tygerberg Strict Criteria are based on observations of the morphology of spermatozoa that have penetrated through cervical mucus (5) and can bind to human zona pellucida (11). The evidence-based 4% using the Tygerberg criteria in the fifth edition of the manual onwards represents an entirely different classification system (12).

The basic methods have been revised in the sixth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen (10). The basic semen examination now focuses on obtaining accurate sperm concentration, motility (including reintroducing the rapid-progressive category), vitality, and morphology (Table 1).

Relationship Between the Mean Percentage of Morphologically Normal Spermatozoa and Male Age

Infertility has become a worldwide problem, affecting up to 20% of couples trying to conceive (15). In this context, a few important facts should be emphasized: an actual decline in semen quality over the past decades has been observed globally (16), and paternal age is rising as more men decide to become fathers at an older age (17). Abnormal standard semen characteristics and reduced sperm chromatin maturity can appear with increasing male age.

Stone et al (18) demonstrated a decline in the percentage of sperm cells with normal morphology in men aged >40 years. Similar results were obtained by other authors (19), who have shown that the percentage of sperm cells with normal structure decreased significantly in men aged >50–79 years.

García-Ferreyra et al (20) studied the association between spermatozoa quality and the age of the male by comparing spermatozoa obtained from men aged ≥40 years (n = 1124) and those with less than 40 years. Their data revealed a decreased semen volume and an increase in the percentage of sperm DNA fragmentation index (DFI) in older men compared to younger men in the entire

Table 1. The Major Changes From the First to the Sixth Edition of the *WHO Laboratory Manual for the Examination and Processing of Human Semen* (14)

Edition	Year	Pages	Major Changes
1st	1980	43	Semen: sample collection, initial examination, sperm motility, sperm density, sperm morphology (plates and stains) Sperm-cervical mucus interaction: collection of mucus, in vitro test, postcoital test
2nd	1987	67	Semen: Standard tests—includes all in the 1st edition + sperm antibody tests Optional tests—semen culture, seminal fluid biochemistry, zona-free hamster oocyte penetration test, sperm migration test Criteria of normality of semen samples
3rd	1992	107	Standard test—use "strict criteria for assessment of sperm morphology." Research tests—zona-free hamster oocyte penetration test, human zona pellucida binding test, acrosome reaction, computer-assisted sperm analysis Sperm preparation Quality control of semen analysis
4th	1999	128	Optional tests—added hypoosmotic swelling test, multiple sperm defects index Research test—reactive oxygen species Quality control—statistical analyses of counting errors
5th	2010	271	Most extensive and comprehensive revision of the semen manual Detailed description of each procedure Added total sperm output per ejaculate as a semen variable Sperm motility combined rapid and slow into one grade of progressive motility Sperm preparations include spermatozoa from the testis and epididymis Using quality control to improve laboratory performance Added chapter on cryopreservation of spermatozoa
6th	2021	276	Step-by-step, easy-to-follow procedure Basic examination—standard tests, reintroduce slow progressive motility Extended examination—optional tests included leucocyte, immature germ cells, added sperm aneuploidy, sperm genetics, and DNA fragmentation Advanced examination—research tests, added membrane ion channels Emerging methods of semen analyses without a microscope Eliminated hamster zona-free penetration test, human zona binding test, and a section on sperm-cervical interaction

study cohort. Moreover, there was a higher incidence of sperm DNA damage (>10% DFI, low fertility potential) in the groups of men aged ≥40 years than in the groups of men aged <40 years. Older men had over twice the odds ratio for high sperm DNA damage as younger men. Their findings suggest a detrimental effect of advanced paternal age on sperm chromatin integrity.

However, several studies (21,22) did not show a relationship between sperm morphology and paternal age. The researchers showed a significantly higher percentage of DFI in the group of older men. Moreover, they found significant correlations between age and the rate of sperm cells with damaged chromatin (23).

A very recent study demonstrated the impact of male age on male reproductive health (The patients were divided into three groups according to their age: Group 1 included male subjects aged 30 years or less, group 2 included male subjects between the ages of 31 and 40 years, and group 3 included male subjects over 40 years of age). The patients in the third group (over 40 years of age) had a higher percentage of sperm chromatin damage (SCD) in their semen. In contrast, conventional semen parameters did not differ statistically ($P > 0.05$) with increasing male age or between different age groups (24).

It is known that a decrease in sperm quality may result from age-related excessive generation of ROS (reactive oxygen species) and sperm-limited antioxidant defenses (25). Additionally, male aging is often associated with defective sperm DNA remodeling mechanisms that result in poorly packaged chromatin and a decreased ability to repair DNA strand breaks. It is, therefore, understandable why older males are more susceptible to oxidative attack and more prone to errors during spermatogenesis (26).

Relationship Between the Mean Percentage of Morphologically Normal Spermatozoa and Ejaculate Abstinence

WHO laboratory manuals for the examination and processing of human semen published since 1980 and the most recently released in 2021 (10) recommend that semen should be collected for semen analysis after a minimum of 2 days and a maximum of 7 days of sexual abstinence, and this instruction has remained unchanged in all these years. However, the European Society of Human Reproduction and Embryology (ESHRE) recommends an abstinence period of only 3–4 days (27).

MacLeod and Gold (28) and Henkel and Schill (29) indicated that the period of abstinence should be based on the frequency of copulation. They reported that a coital frequency of less than three times per week could result in delayed fertility due to a missing ovulatory window and/or impaired sperm parameters (29).

A systematic review and meta-analysis, which investigated the impact of a very short abstinence period on sperm parameters and the SDF rate, suggested that a second ejaculation collected after a very short period from the first one contains spermatozoa of better quality, in terms of sperm concentration, total and progressive motility, and the SDF rate in patients with abnormal sperm parameters (30). These results could have significant implications in both natural and ARTs.

Various studies (n=16) evaluated the effects of abstinence time on morphology, using either Kruger's strict criteria or the WHO criteria for evaluating morphology. Eleven studies did not show significant differences in morphology with varying abstinence times in healthy men or those with suspected infertility. No clear consensus was apparent regarding an ideal abstinence time to maximize morphology (31).

Borges et al reported that ejaculatory abstinence of four days or less was associated with lower SDF and higher rates of fertilization and pregnancy compared to longer ejaculatory abstinence in couples undergoing ART (32). Indeed, a higher percentage of progressive sperm motility and lower levels of SDF were reported in a short abstinence cohort. Otherwise, the extended abstinence group reported higher sperm concentrations (33).

The percentages of DNA fragmentation and MMP (mitochondrial damage) worsened with the increased duration of abstinence. The rate of sperm protamination was statistically significantly increased with abstinence. However, semen pH, morphology, and apoptosis percentage did not change significantly (34).

Despite accumulating evidence, the WHO recommends a minimum of two days and a maximum of seven days of abstinence (10). This wide range should be considered when interpreting sperm quality.

Relationship Between the Mean Percentage of Morphologically Normal Spermatozoa and Heat Stress

Another major factor that may contribute to male infertility is exposure to excessive heat at the workplace or due to climate change. Temperature plays a crucial role in maintaining normal spermatogenesis in the testes. The scrotal temperature is 2–4 °C lower than the core body temperature (35). Furthermore, it was observed that a 1–1.5 °C elevation in scrotal temperature can result in impaired sperm production (oligozoospermia, azoospermia, and sperm morphological abnormalities (teratozoospermia) (36).

Furthermore, various animal studies have also shown that a rise in testicular temperature results in reduced testicular size, decreased sperm production, increased abnormal sperm morphology, and reduced motility, leading to male infertility (37). Hence, exposure to high temperatures, both due to occupation and environmental factors, has a deleterious impact on overall semen quality and can cause male infertility (38).

Besides, environmental stresses, such as a temperature rise, could activate heat shock protein (HSP). HSP70s is one of the major classes of proteins induced by elevated temperatures. They are responsible for the folding,

assembling, and disassembling of other proteins (39) and are known to play a crucial role in spermatogenesis (40). Hence, any factor that perturbs their regular expression and regulation adversely impacts male fertility (41).

Relationship Between the Mean Percentage of Morphologically Normal Spermatozoa and Sperm DFI

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 (5). Some specific defects (affecting 99% or 100% of spermatozoa) are also associated with genetic abnormalities, such as globozoospermia, sperm macrocephaly syndrome, multiple tail abnormalities, or headless spermatozoa.

The sperm DFI reflects the integrity of and damage to the DNA and genetic material of the sperm, thereby detecting potential sperm damage. It is considered a crucial indicator in evaluating semen quality. Sperm DNA fragmentation (SDF) impacts fertilization, embryonic development, and paternal genetic information transmission during spontaneous and ART pregnancies (42).

SDF occurs during spermatogenesis and maturation, producing broken DNA fragments in sperm cells due to damaged chromosomes and impaired DNA integrity (43). The sperm DFI is used to assess the DNA damage and directly reflects the degree of sperm DNA destruction. Human sperm DNA carries the paternal genetic information, and its integrity is required to transmit genetic materials to the offspring correctly. Damage to sperm chromatin can directly affect the sperm's normal functions (44).

The following three major factors cause sperm DNA damage: abnormal sperm chromatin assembly, aberrant apoptosis of sperm cells, and excessive oxidative stress (45). During sperm maturation, histones are gradually replaced by the smaller arginine- and cysteine-rich protamine (HP). This process reduces the ability of sperm DNA to repair itself in response to changes in the internal and external environments. Furthermore, the misfolding of DNA supercoiling structures in the chromosome due to twisting tensions generated by the double-stranded DNA helix can also lead to aberrant DNA repair, causing SDF or abnormalities in the chromatin structures (46).

Yang et al (47) show clinical pregnancy rates following IUI among high, medium, and low sperm DFI groups were 12.5% (11/88), 14.3% (48/336), and 13.4% (102/761) and no statistical difference between the groups (P<0.88) could be found. However, early abortion rates among these groups were 27.3% (3/11), 14.6% (7/48), and 4.9% (5/102), showing these differences in abortion rates between the investigated groups were statistically significant (P< 0.02). However, no significant differences in the rates of clinical pregnancy, early abortion, oocyte fertilization, or good-quality embryos in in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles were detected among different DFI groups (P<0.05).

The relationship between DFI and sperm morphology was investigated in a retrospective study. The DFI-morphology correlation was observed only in the motile sperm population identified after the swim-up performed to select the spermatozoon subsequently used in ICSI or IVF procedures (48).

The authors concluded that in case of detection of DFI ≥ 15% in the whole semen sample, the DFI analysis should be performed in spermatozoa selected after swim-up to avoid picking out a spermatozoon presenting a normal morphology but a fragmented DNA from the pellet. This result suggests that a spermatozoon with a normal morphology could exhibit high DFI, causing a reduced embryo quality and pregnancy rate after ICSI (49).

Two studies, a French prospective study (n=1633) (50) and a Chinese retrospective survey (n=1790) (51), analyzed the correlation between DNA damage of spermatozoa and other sperm parameters and demonstrated that both progressive motility and normal morphology were inversely correlated to the rate of DNA damage. However, no correlations were found between the DFI and sperm concentration, age, tail defects, and abstinence time.

Another study, applying the Halosperm test and hypo-osmolality swelling test (HOS-test), also revealed a significant negative correlation between sperm morphology and DFI (52).

Relationship Between the Percentage of Normal Forms and Lifestyle

Different studies have shown that semen parameters may be affected by various lifestyles, advancements in technology, environmental pollution (53), smoking and alcohol intake (54,55), psychological stress (56), Obesity, and dietary (57).

Smoking and Alcohol

Several studies suggest a strong correlation between smoking and altered semen parameters (58). It has been shown that moderate exposure to heavy metals found in cigarettes, especially cadmium and lead, affects male reproductive and endocrine functions by decreasing human semen significantly, thus impairing male fertility (59). Other chemicals in tobacco smoke that cause damage to the cells are tar, nicotine, CO, and hydrocarbons, such as polycyclic aromatic hydrocarbons, some radioactive compounds, and toxic heavy metals (60).

In addition, cigarette smoking can increase inflammatory reactions, resulting in increased levels of leukocytes in the testicles (61). Fragmentation of the sperm DNA, axonemal damage, and decreased concentrations of sperm cells have also been observed among smokers (54,55).

Various studies conducted to determine the relationship between smoking and semen parameters. The results indicated that the semen parameters were significantly higher in non-smokers than in smokers. Also, semen volume and sperm vitality were considerably higher in the group of non-smokers in comparison to heavy smokers ($P < 0.037$ and $P < 0.035$, respectively). The same was noticed for total motility, morphologically normal spermatozoa mean percentage, and membrane integrity ($P < 0.0001$). However, protamine deficiency (CMA3) and DNA fragmentation (TUNEL) were significantly higher in smokers than in non-smokers ($P < 0.0001$) (55-58).

Besides, alcohol is also known as a dietary factor that affects fertility by giving rise to the production of metabolites like acetyl and methyl radicals, which are responsible for ROS generation. Also, regular alcohol consumption triggers lipid peroxidation, increasing ROS production, protein degradation, and DNA fragmentation (62). It also lowers SOD antioxidant activity, along with GSH levels (63).

However, the studies on couples undergoing ART or any of the infertility treatments remain controversial (64). Earlier studies showed the deleterious effects of alcohol (65). Following these studies (66) demonstrated no association between fertility and alcohol consumption.

Obesity

Persons with obesity have augmented estrogen levels due to the amplification of aromatase in the adipose tissue; through a negative response loop, men display indications of hypogonadotropic hypogonadism. Besides augmented oxidative stress, lipotoxicity, and instabilities in the absorptions of adipokines, these hormonal fluctuations directly distress the gonads, peripheral reproductive organs, and the embryo (67).

It is generally well-accepted that reproductive function highly correlates with the degree of adiposity, nutrition, or metabolic condition related to human food intake (68). Paternal BMI $kg/m^2 < 16.5$ (underweight) and > 30 (obesity) were associated with reduced semen quality (69). Similarly, a direct association was found between men's BMI kg/m^2 and semen quality even after adjustment for reproductive hormones (70).

Bibi et al (24) found no influence of paternal BMI on sperm morphology and concentration, while overweight men had lower motility compared to normal-weight men. Similarly, researchers observed that increased paternal BMI leads to lower fertilization and clinical pregnancy rates after the ART cycle.

Other factors

Other factors, known as acquired factors that contribute to male infertility include infection, immunological factors, trauma or surgical insult to the male reproductive organs, and exposure to toxic chemicals or other materials (71).
Hence, chromatin condensation and DNA integrity are correlated with negative fertility consequences, which might be usually characterized by low fertilization rates, bad embryo quality, repeated failures of ART attempts, and miscarriages (72). IVF and ICSI have greatly helped subfertile couples to conceive, but the success of these technologies depends on the semen parameters and sperm DNA quality (73).

Relationship Between the Mean Percentage of Morphologically Normal Spermatozoa and ART Outcome

For human ARTs, the morphology, size, and acrosome of sperm heads are essential criteria for sperm selection. Recent studies have shown a correlation between sperm head size, shape, chromosomal abnormalities, and fertilization rate (74).

Kruger et al (13) published strict criteria for morphological evaluation of human sperm (Figure 1). Under these criteria, a good sperm has an elliptical or barrel-shaped head, a straight midpiece that is neither swollen nor thickened, a straight tail, and an acrosome occupying 40%–70% of the sperm head (13). Furthermore, normal sperm have a head length of 4.1 µm (range 3.7– 4.7 µm), width of 2.8 µm (range 2.5–3.2 µm), and an aspect ratio of 1.5 (range 1.3–1.8) according to WHO guidelines (10).

Indeed, a direct correlation between fertilization rate and semen parameters (sperm count, motility, and morphology) is detected in both ICSI and IVF cycles, and fertilization rate is related to sperm morphology in ICSI cycles and to sperm motility in IVF cycles (75).

A retrospective study (76) analyzed 427 and 2,728 cycles according to the mean percentage of morphologically normal spermatozoa <4% and ≥4% group, respectively. The total fertilization failure, implantation, abortion, clinical pregnancy, and neonatal (sex, gestational age, preterm birth, birth weight, low birth weight, live births, and congenital disabilities of newborns) outcomes were compared. Total fertilization failure in the group of sperm morphology <4% group was significantly higher compared to that in the normal sperm morphology ≥4% group (2.8% versus 1.2%, $P = 0.012$)

However, the implantation and abortion rates and clinical pregnancy were not significantly different between the two groups. Additionally, the sex, preterm birth, low birth weight, live births, congenital disability rates, gestational age, and birth weight of newborns were not significantly different between the two groups (76).

Another study (77) showed that couples with teratozoospermia had a significantly lower optimal embryo rate compared to those with normal sperm morphology in IVF ($P = 0.007$), while there were no statistically significant differences between the two groups (teratozoospermia and normal sperm) in terms of the fertilization rate, cleavage rate, implantation rate, and pregnancy rate ($P > 0.05$). Additionally, teratozoospermia

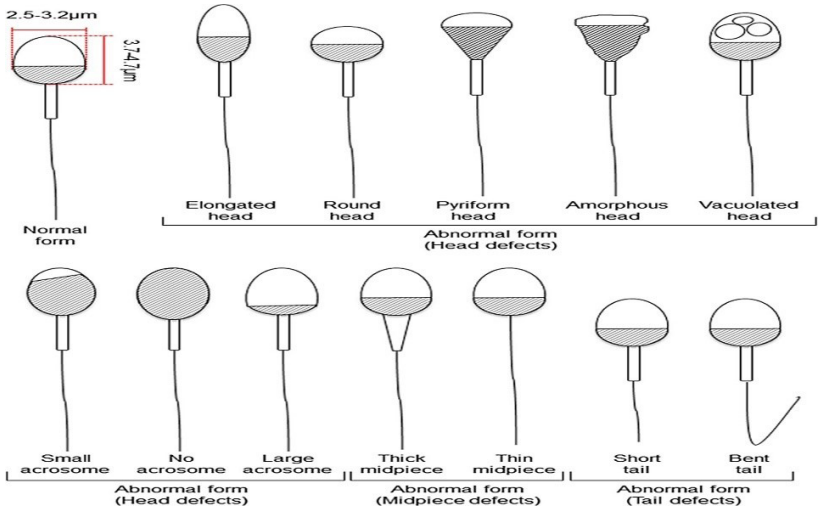

Figure 1. Schematic Diagram of Human Sperm Showing Normal and Abnormal Forms Based on Kruger's Strict Criteria (13) and WHO Criteria (10).

was associated with lower infant birth weight in multiple births after IVF. Concerning ICSI, there was no significant difference in both pregnancy outcome and newborn outcome between the teratozoospermia and normal groups (both $P > 0.05$). Furthermore, no increase in the risk of congenital disabilities occurred in the teratozoospermia group after IVF/ICSI (77). Many studies have shown controversial results of pregnancy outcomes in patients with teratozoospermia undergoing ART. Evidence from recent research has suggested that there was no difference in the IVF/ICSI outcome among men with teratozoospermia.

Evidence suggests that the role of sperm in embryogenesis goes beyond genomic material transfer, and centrosomes, sperm-derived cytoplasmic factors, paternal mRNA, and small RNAs are essential for early embryonic development. Epigenetic factors like histone modification and DNA methylation participate in the regulation of gene expression in sperm. Nevertheless, the etiology of sperm chromatin abnormalities is essential in male fertility and may affect reproductive outcomes. Implantation success depends not only on the sperm and oocyte quality but also on the type of ARTs. Therefore, male factors affect embryo development and can play a crucial role in the failure or success of ARTs (78).

On the contrary, Yang et al demonstrated that IVF/ICSI outcomes are not related to sperm DFI and found that elevated sperm DFI does not impact oocyte fertilization or embryo development (47). A previous study (46) found that sperm DNA damage contributes to a negative predictive factor for couples undergoing ART. Nonetheless, Horta et al (79) pointed out that high levels of SDF may be corrected by the repair mechanism of oocytes, especially those from younger females, and therefore no effect of sperm quality on embryo development (79).

It is generally accepted that sperm morphology is not related to ICSI outcome because of the selection of optimal sperm and bypasses both zona pellucida binding and penetration (77).

The role of sperm morphology in IVF/ICSI remains open to debate. We believe that sperm morphology has limited predictive value for pregnancy outcomes in IVF/ICSI.

In addition, lifestyle modifications, including cessation of smoking, exercise in moderation, maintaining a healthy diet, an ideal body mass index, prompt treatment of testicular inflammation, genital tract infections, and varicocele corrective interventions, may each benefit such cases (80). Various antioxidant therapies given empirically are beneficial for infertile men (81).

Conclusions

Many authors have endeavored to study human sperm morphological abnormalities. They highlighted correlations between the percentage of morphologically normal spermatozoa, some sperm functional abnormalities, lifestyle, and spontaneous fertility. What makes spermatozoa successful in reaching the site of fertilization and fertilizing the egg depends on some traits (good motility, adequate morphology, and normal DNA status). Sperm heterogeneity in an ejaculate may have functional relevance, ensuring a greater potential to fertilize after being deposited in the female genital tract. Sperm morphology is the most relevant parameter in conventional semen analysis for predicting fertilization potential. We believe the role of sperm morphology in IVF/ICSI remains open to debate.

Authors' Contribution

Conceptualization: D Shennou
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Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

Not applicable.

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