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Relationship between spermatogenesis, DNA fragmentation index, and teratozoospermia index

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***Abstract*---**Infertility has proven a global medical issue, with 15-20 percent of married worldwide experiencing difficulties in conceiving. One of the causes of male infertility is sperm morphology abnormalities, or teratozoospermia, which is often associated with sperm DNA damage. Teratozoospermia can increase sperm DNA fragmentation through several mechanisms, including abortive apoptosis and induce ROS formation. Unfortunately, many forms of sperm flaws, including genetic defects, could not be validated by routine semen examination. In the Sixth Edition, WHO introduced a more thorough sperm morphology examination, named the teratozoospermia index (TZI), in which every aberrant sperm is examined for up to four defects. Analyzing spermatozoa abnormalities in more detail can provide an overview of the function of the male

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reproductive organs, particularly the testes and epididymis. However, using this method is not entirely easy due to subnormal variations between cells so that quality control may be needed when using TZI for sperm morphology evaluation.

***Keywords*---**male infertility, spermatogenesis, sperm DNA fragmentation, teratozoospermia index.

Introduction

Infertility has proven a global medical issue, with 15-20 percent of married worldwide experiencing difficulties in conceiving, according to Candela et al. (2021, p.4). Around 20–70% of infertility instances are caused by male variables, with nearly 30% of these variables directly influencing fertility (Candela et al., 2021, p.5). One of the causes of male infertility is sperm morphology abnormalities, which is often associated with sperm DNA damage. Traditional semen testing, as defined by WHO guidelines, is the initial step in determining male infertility. According to Jakubik-Uljasz et al. (2020, p.8), many forms of sperm flaws, including genetic defects, could not be validated by standard examination. This semen study has limited therapeutic utility in evaluating sperm reproductive ability. As a result, additional diagnostic methods are needed to supplement the traditional cement examination. The study shows the relationship between spermatogenesis, DNA fragmentation index (DFI), and teratozoospermia index (TZI).

The creation and maturation of spermatozoa inside the male reproduction organ, the testicles, is known as spermatogenesis. The testicles are made up of many thin, tightly wound glomeruli called seminiferous tubules, which fabricate spermatozoa within their sidewalls. In addition, the DFI is a statistic computed by dividing the figure of sperm motility with fragmented DNA by the overall frequency of sperm cells tested. Lastly, the TZI is an excellent determinant of conception and motility. It is a numerous abnormalities index in which 100 sperm cells are recorded incompletely. The quantity of conventional and defective sperm is maintained on track, and every aberrant sperm is examined for up to four defects.

Male fertility could be determined by sperm morphological defects and aberrant sperm genome completion, according to Yang et al. (2019, p.11). As a result, the study is essential since it assesses sperm morphological defects and sperm DNA destruction, which is critical for determining male fertility ability. Nevertheless, since sperm morphogenesis, which includes high chromatin compression in spermiogenesis step, is distinct and complicated, these illnesses' pathophysiology and linkages are not entirely recognized (Santi et al., 2018, p.11; Atshan et al., 2020, p.6). These steps result in the formation of particular sperm components necessary for oocyte implantation. Atshan et al. (2020, p.13) argue that morphogenesis deficiency could result in gamete's nuclear DNA fragmentation due to defective healing of nuclear genomic instability throughout the initial spermatogenesis phase.

Moreover, sperm with developmental disturbance have been shown to produce more responsive oxygen radicals and possess more outstanding DNA breakage (Arumugam et al., 2019, p.9). Possible outcomes are reactive damage, 'ultimately unsuccessful' death of specialized germinal tissues, and teratozoospermia. The presence of aberrant compacted chromatin and degraded nuclear DNA with sperm head abnormalities in ejaculate must be noted (Chopra et al., 2021, p.16; Siddhartha et al., 2019, p.18). It is also impossible to say whether structural abnormalities cause gamete's nuclear DNA thread fractures or if chromatin reconfiguration failures encourage the production of aberrant sperm head abnormalities; it is only safe to presume that both causes are present (Das et al., 2022, p.31). One study by Das et al. (2022, p.29) was try to examine the link involving sperm DNA and sperm morphology distribution, as well as to identify the occurrence of various sperm DNA fragmentation (SDF) values in males with teratozoospermia and to construct a sperm morphology differentiated specified limit for SDF (Figure 1).

Comparing morphology based on normal and abnormal spermatozoa forms

Sperm morphology can be seen from the size and shape of the spermatozoa. To assess ideal sperm morphology, it is necessary to evaluate four main parts: head, midpiece, tail, and the presence of excess residual cytoplasm. Ideal spermatozoa morphology criteria, along with abnormal condition examples, can be seen in Table 1. More specifically, teratozoospermia is diagnosed when more than 95% of the spermatozoa in semen sample have abnormal morphology (WHO, 2021). Teratozoospermia is the result of defective cell differentiation during spermatogenesis and has been associated with several genetic and environmental factors as well as advanced paternal age and psychological stress (De Braekerleer et al., 2015; Coutton et al., 2015). Many studies have shown that semen samples with teratozoospermia generally result in lower fertilization rates (Zhou et al, 2021). However, this is very subjective as it depends on the examiner.

Table 1
Normal and Abnormal Human Spermatozoa Measurement (WHO, 2021; Lasiene, 2018; Maree, 2010)

Features	Measurement	Abnormal Examples
Head	Length 4-5 m, width 2.5–3.5 m, length-width ratio 1.5–1.75 Papanicolaou: length: 4.28 ± 0.27m, widths:2.65 ± 0.19m; length-width ratio: 0.62 ± 0.04m Rapidiff: length: 5.17 ± 0.27m; widths:3.12±0.21m; length-width ratio: 0.60 ± 0.04 SpermBlue: length: 4.73 ± 0.27m; widths:2.75±0.24m; length-width ratio: 0.58 ± 0.04 Fresh measurement: length: 4.79 ± 0.26m; widths:2.82 ±	1. Acrosome less than 40% or larger than 70% of a normal head area 2. Length-to-width ratio less than 1.5 (round) or larger than 2 (elongated) 3. Shape: pyriform (pear shaped), amorphous, asymmetrical, or non-oval shape in the apical part 4. Vacuoles constitute more than one fifth of the head area or located in the post-acrosomal area

	0.23m; length-width ratio: 0.59 ± 0.04	5. Double heads 6. Any combinations
Midpiece	Length 7–8 m, width < 1 m	1. Irregular shape 2. Thin or thick 3. Asymmetrical or angled insertion at head 4. Sharply bent 5. Any combinations
Tail	Length 45–50 m, tail/head length ratio 10.3+/-0.2	1. Sharply Angulated Bends 2. Smooth hairpin bends 3. Coiled 4. Short (broken) 5. Irregular width 6. Multiple tails 7. Any combinations
Cytoplasmic residue	Cytoplasmic droplets (less than one third of a normal sperm head size) are normal	Residual cytoplasm is considered an anomaly only when it exceeds one third of normal sperm head size

To determine teratozoospermia with TZI, spermatozoa are carefully examined for the number of defects in each section. TZI can range from 1 to 4. For example, TZI 1 indicates defects in one part that occurs most frequently, index 2 means defects in two parts, and index 3 means defects in three parts (WHO, 2021; Gatimel et al., 2017). Thus, this index helps in detecting the severity of sperm morphological defects. Assessment of spermatozoa morphology is fundamentally important in terms of male fertility prognosis and ART outcome. However, analyzing spermatozoa abnormalities in more detail can also provide an overview of the function of the male reproductive organs, particularly the testes and epididymis. Therefore, it is important to evaluate any abnormalities in the spermatozoa, starting from the head, neck, tail, and the presence or absence of excess residual cytoplasm (WHO, 2021).

Possible association between DFI and TZI

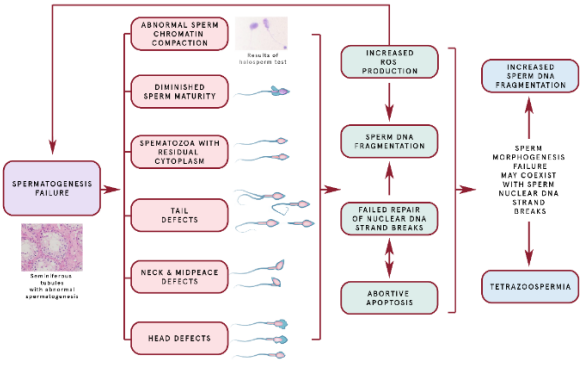

Figure 1. Relationship of Spermatogenesis and teratozoospermia and DNA Fragmentation Index

The above flowchart (Figure 1) indicates the theoretical process which can be used to understand the link between spermatogenesis, DFI, and TZI. The theorized process causes both sperm chromatin disorders and sperm morphological flaws simultaneously. Boeri et al. (2020, p.24) state that abnormal fertilization can cause an upsurge in sperm morphology defects, contributing to teratozoospermia, sperm maturation issues, and sperm chromatin consolidation problems. It also leads to the inability of embryological cells to complete apoptosis and nuclear DNA fragmentation to be repaired (Boeri et al., 2020, p.24). It indicates that blastocyst cells are resistant to apoptosis and possibly show genomic nuclear instability. Immature sperm cells release more superoxide radicals, leading to sperm genomic instability and spermatogenesis dysfunction.

According to Khamar et al. (2020, p.41), SDF was negatively correlated with sperm density, overall sperm measure, sperm morphological characteristics, gradual sperm cell migration, and overall gametes progressive sperm. On the other hand, a positive relationship was found between SDF and the TZI, proportions of interstitial fluid spermatogenesis, head faults, set of particular defects, tail deformities, and the proportion of sperm with remaining cytoplasm (Khamar et al., 2020, p.54). Researchers compared the data from males with teratozoospermia to individuals with standard sperm morphology to see a correlation between sperm structural deficits and sperm nuclear DNA breakage (Wang et al., 2022, p.13). The findings showed that sperm morphological abnormalities and aberrant nuclear spermatogenesis dispersion existed. According to Fistand Ibrišimović (2019, p.13), results demonstrated that males differed significantly in essential sperm concentration, comprehensive semen morphological features, and SDF frequency as determined by the Halosperm exam (Tinjić and Ibrišimović, 2019, p.17). Males with teratozoospermia showed a more significant portion of ejaculate with poor chromosomal coherence and a greater proportion of severe sperm physical abnormalities.

In the current sample of males with aberrant sperm quality, 32.77 percent of the participants had a more excellent standard of SDF, whereas 4.84 percent of the men with sperm production morphological features had a minimum concentration of SDF (Sakhil et al., 2021, p.32). Panner et al. (2020, p.15) say that decreased amounts of SDF were identified in just 22.97 percent of males with teratozoospermia and sometimes even 58.60 percent of men lacking a negative association. These findings match the OR, which showed that men with aberrant sperm quality had a nearly ten-fold greater probability of having an SDF concentration >30% than males with normal spermatogenesis structure (Opuwari et al., 2019, p.11).

Our findings were somewhat consistent with data collected by other researchers. Opuwariet al. (2019, p.15) provided a clear link between spermatogenesis and sperm quality in their research. The study found that a thorough morphological inspection is critical throughout regular cement assessments. It could be an excellent forecast of poor sperm quality in the experimental examination of sterile men when combined with DNA stability indicators (Ammar et al., 2019, p.39). Another group of researchers discovered that SDF was significantly greater in teratozoospermia patients than in the control group, implying a link between aberrant sperm shape and SDF. Moreover, immature and/or pathological

spermatozoa can cause excessive levels of reactive oxygen species (ROS). Spermatozoa are susceptible to ROS-induced damage because of a membrane structure rich in polyunsaturated fatty acids (PUFA) that surrounds the head and mitochondria of spermatozoa. At the molecular level, ROS can directly damage the sperm DNA by attacking purine and pyrimidine bases and can also initiate the apoptotic pathway, activating caspase enzymes to degrade sperm DNA (Cho et al., 2016).

Optimal DFI cut-off value

One study indicated a significant negative correlation between sperm density, total sperm quality, sperm morphology, gradual and sperm count spermatogenesis, and sperm strength, but favorable relationships between variables with the TZI (Kaewman et al., 2021, p.16). Other writers have found similar relationships, and it should be emphasized that semi-logical research seldom tries to find connections between precise spermatogenesis and seminal chromatin maturation. Only Ammaret al. (2020, p.19) discovered a link between the proportion of irregular sperm motility and SDF along with connecting head deformities and fractured DNA.

According to Wang et al. (2019, p.14), researchers created a receiver operating characteristic (ROC) curve to determine whether there's a link between sperm nuclear DNA breakage and sperm structure. The most important criteria for identifying normal and defective sperm shape was the proposed ideal SDF level of 18 percent evaluated using the DNA male scattering test (Shi et al., 2019, p.32; Xie et al., 2020, p.41). According to the findings, 62.84% of teratozoospermia patients had SDF values of >18% because they exhibited a five-fold more significant chance compared to males lacking the disease (Maia, 2020, p.31). To their understanding, this is the initial research to use ROC to establish SDF's predictive ability for sperm parameters. However, most experts believe that these two characteristics are connected and crucial in infertility diagnosis. DNA fragmentation is the principal mechanism contributing to structural abnormalities in sperm, according to Ammar et al. (2019, p.12). In addition, reduced chromatin compression, which can produce DNA fractures and damage the DNA spine, seems to be an independent consequence of poor sperm count morphology.

Geneva et al. (2021, p.9) revealed that a significant proportion of spermatogenesis with head malformations in sperm specimens implies a substantial level of DNA breakage. They also showed that if the researchers utilized a 20% cut-off number for strand breaks, there was a considerable variation in the proportion of spermatozoa, with showed standard among sperm specimens with strand breaks and sperm specimens with high strand breaks (Ganeva et al., 2021, p.18). For instance, in the sample with a 20% level of strand breaks, the proportions of macrocephalic, anencephaly, lengthened heads, nuclear deformities, nebulous heads, and tail deformities were greater.

A quantitatively significant effect was found once all head deformities were considered combined. According to Saito et al. (2020, p.39), disruptions in spermatogenesis generation and development might significantly affect the

current properties of gametes. They stress that sperm structure is the essential criterion for conventional semen examinations since it details implantation capability (Mehrparvar et al., 2020, p.28; Abdel et al., 2020, p.34). Bin Azizan (2018, p.34) says that in the experimental evaluation of infertility males, sperm structure, in conjunction with current indicators of semen parameters, may offer the most significant indicator values for poor sperm concentration. Consistent with their hypothesis that males with teratozoospermia are at a greater risk of seminal strand breaks, according to Saito et al. (2020, p.41).

Limitations of TZI

Basically, TZI is an excellent measuring tool for assessing male reproductive organs and ART outcomes. However, sperm morphology assessment by section is not entirely easy due to subnormal variations between cells. Some of the variability is linked to the continuous nature of sperm shape and size, and thus cannot be eliminated, making the classification of subnormal shape and size difficult (Augers, 2010). Thus, quality control may be needed when using TZI for sperm morphology evaluation. In addition, the relationship between TZI and DFI still needs to be investigated because of limited publications regarding this matter.

Conclusion

In conclusion, this review time to check the relationship between spermatogenesis, DNA fragmentation index, and teratozoospermia index. Thorough evaluation of each part of the spermatozoa is important for teratozoospermia diagnosis. There is little question, nevertheless, that the Halosperm study's proposed ideal result of 18% is a criterion for predicting standard and defective sperm structure, indicating the immediate treatment, and valuable for executing the appropriate treatment situation for males with teratozoospermia. Comprehensive sperm structural characteristics deficiencies find common ground with unusual nuclear spermatozoa scattering. Males with teratozoospermia could be at a greater peril for gametes strand breaks. The intended SDF significance of 18% evaluated using the DNA gametes scattering test is the most satisfactory characteristic for predicting healthy and unhealthy sperm morphology.

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