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Assessment of Sperm Morphometry in Evaluating Male Infertility

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Abstract

Background: Infertility is a complex issue affecting 15% of couples of reproductive age, with men accounting for 40%-50% of infertility cases. Semen analysis comprises various descriptive measures of sperm and seminal fluid to determine semen quality. Transforming qualitative descriptions of sperm deformities and shape changes into quantitative terms can aid in identifying sub-visual abnormalities. This study aimed to evaluate sperm morphometry parameters in both infertile and fertile men.

Methods and Results: The study enrolled a total of 101 participants, divided into three groups: Group A included 38 subfertile patients with varicocele, Group B included 33 patients with idiopathic infertility (23 with asthenozoospermia and 10 with oligozoospermia), and Group C (the control group) included 30 healthy fertile men. The mean age of patients was 31.6±5.81, 31.3±6.0, and 29.47±4.27 years in Groups A, B, and C, respectively (P>0.05). Scrotal duplex examinations were performed to identify the presence of varicocele. Semen samples were collected following WHO Manual (2010). Semen dynamic and morphological analyses were conducted using CASA (Computer-Assisted Semen Analysis, MIRALAB, ISO9001, ISO13485). We found that sperm concentration, total sperm count, sperm progressive motility, and sperm progressive+non-progressive motility were significantly lower in Group A and Group B than in Group C (P=0.000 in all cases); however, there were no differences between Group A and Group B regarding these parameters. The sperm morphology index was significantly lower in Group A than in Group C (P=0.0024); no differences were found between Group B and Group C and Group B and Group A. The mean value of the sperm deformity index was significantly lower in Group A than in Group C (P=0.004).

Conclusion: Our study highlights the significant association between sperm morphology and male infertility in varicocele and idiopathic subfertile males.**(International Journal of Biomedicine. 2024;14(1):93-98.)**

Keywords: infertility • semen quality • varicocele

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Abbreviations

MAI, multiple anomalies index; **TZI**, teratozoospermia index; **SDI**, sperm deformity index.

Introduction

Infertility is a condition of the reproductive system characterized by the inability to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual activity. Infertility is a complex issue affecting 15% of couples of reproductive age,[1] with men accounting for 40%-50% of infertility cases.[2,3] Various factors, including occupational hazards, exposure to reproductive toxicants, chemotherapy, radiation therapy, heat exposure, physical labor, lifestyle variables (wearing tight underwear, poor diet), genital injuries, hereditary traits, testicular maldescent, infections, and iatrogenic causes, can contribute to decreased male fertility.[4-6]

The most prevalent form of male infertility is idiopathic male infertility, which is characterized by the presence of one or more abnormal semen parameters without a clear explanation.[7] Following closely is varicocele, accounting for 35% to 50% of men with primary infertility and up to 81% with secondary infertility.[8] The negative impact of varicocele on spermatogenesis can be attributed to several factors, including elevated testicular temperature, increased intratesticular pressure, hypoxia due to reduced blood supply, reflux of toxic compounds from the adrenal glands, and hormonal profile abnormalities.[9,10]

Semen analysis involves a set of descriptive measurements of spermatozoa and seminal fluid parameters used to assess semen quality.[11] Determining sperm morphology, however, poses challenges due to subjective factors and inconsistency. A comprehensive assessment of sperm shape necessitates an evaluation of the head, neck, midsection, and tail. In normal sperm, the head should be oval and symmetrical, and tail insertion should be axial, in line with the long axis of the head. Abnormal sperm variations include those with oversized, undersized, round, asymmetrical, or amorphous heads, as well as those with tapering, bulging midpieces, multiple heads or tails, or amorphous heads.[12] Typically, clinical laboratories apply sperm morphology parameters established by the WHO or the "strict morphology" criteria developed by Dr. Kruger.[13]

To enhance the quantitative identification of sperm shape, morphometric methods to measure sperm under normal conditions can establish a reference for quantitative terms, replacing qualitative descriptions with more precise numerical terms. Quantitative stereological methods allow investigators of seminal samples to derive three-dimensional concepts from two-dimensional microscopic fields. This enriches the biophysical assessment of sperm by calculating absolute and relative volumetric parameters, which conventional microscopic assessment cannot provide.[14,15] Converting qualitative descriptions of sperm deformities and shape changes into quantitative numerical terms can be particularly valuable in identifying sub-visual shape changes and abnormalities.[16,17] Using quantitative numerical descriptions for qualitative characteristics can facilitate the comparison of different treatment modalities and determine their respective advantages. Mathematical descriptions of sperm movement allow for a more precise expression of the type of movement and velocity, which can be challenging to convey using ambiguous qualitative terms.[18,19]

A recent study by Rrumbullaku et al.[20] demonstrated a significant increase in the percentage of tapered spermatozoa, spermatozoa containing cytoplasmic droplets, and spermatozoa with bent tails in varicocele patients, compared to controls. In our study, we evaluated sperm morphometry in varicocele, non-varicocele infertile patients, and controls using Computer-Assisted Semen Analysis (CASA, MIRA LAB, ISO9001, ISO13485). This approach promises to provide a more accurate and quantitative assessment of sperm morphology, shedding light on potential sub-visual abnormalities and shape changes that could be contributing to male infertility.

This study aimed to evaluate sperm morphometry parameters in both infertile and fertile men.

Materials and Methods

Study Setting and Participants

This prospective study took place at the Andrology Unit of Alazhar University Hospital (Assiut) and was conducted with the approval of the relevant authorities. Informed consent was obtained from all participants. The study enrolled a total of 101 participants, divided into three groups: Group A included 38 subfertile patients with varicocele, Group B included 33 patients with idiopathic infertility (23 with asthenozoospermia and 10 with oligozoospermia), and Group C (the control group) included 30 healthy fertile men.

Data Collection

Participants underwent a comprehensive assessment, including the following aspects:

History: This included information such as patient age, age of puberty onset, age of varicocele onset (if applicable), sexual history, number of children, lifestyle habits (smoking, alcohol, drug use), medical history (using cytotoxic, teratogenic, or antiandrogen drugs), surgical history, spinal cord trauma, prostatectomy, sexually transmitted diseases, and epididymitis or epididymo-orchitis.

Examination: General and genital examinations were conducted, encompassing secondary sexual characteristics, body musculature, tall span index, gynecomastia, and body mass index, as well as a thorough examination of the penis, scrotum, epididymis, vas deferens, and spermatic cord.

Scrotal Duplex: Scrotal duplex examinations were performed to identify the presence of varicocele.

Semen Analysis: Semen samples were collected following WHO Manual (2010), with a recommended abstinence period of 2-5 days. Samples were collected by masturbation in sterile containers without the use of lubricants or soap. Samples were incubated at 37°C until complete liquefaction occurred (30-60 minutes). Dynamic and morphological analyses were conducted using CASA (Computer-Assisted Semen Analysis, MIRALAB, ISO9001, ISO13485) to assess sperm parameters.

Exclusion Criteria: Patients with conditions such as erectile dysfunction, benign prostatic hyperplasia, psychological disorders, genetic sex disorders, azoospermia, necrozoospermia, severe debilitating diseases, malnutrition, or use of cytotoxic, teratogenic, or antiandrogen drugs were excluded from the study.

Statistical analysis was performed using the statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY:

IBM Corp). For the descriptive analysis, results are presented as mean (M) ± standard deviation (SD). Multiple comparisons were performed with one-way ANOVA and Tukey HSD post-hoc test. Group comparisons with respect to categorical variables are performed using chi-square tests A probability value of $P<0.05$ was considered statistically significant.

Results

The mean age of patients was 31.6±5.81, 31.3±6.0, and 29.47±4.27 years in Groups A, B, and C, respectively ($P>0.05$). Primary infertility was diagnosed in 27(71.1%) patients of Group A and 21(63.6%) patients of Group B (Table 1). In Group A, the majority of patients had varicocele grade II (55.3%), followed by grade III (39.5%) and grade I (5.3%) ($P=0.0006$) (Table 2). In Group B, 10(30.3%) patients had oligoasthenozoospermia, and 23(69.7%) patients had asthenozoospermia.

Table 1.

Type of infertility in the patients of the study groups.

Type of infertility	Group A (n= 38)	Group B (n=33)	Statistics
Primary	27 (71.1%)	21 (63.6%)	$\chi2=0.444$ df=1 P=0.505
Secondary	11 (28.9)	12 (14.1%)	

Table 2.

Distribution of patients in Group A according to the varicocele grade.

Grade of varicocele	Group A (n=38)	Statistics
Grade I	2 (5.3)	$\chi2=14.895$ df=2 P=0.0006
Grade II	21 (55.3%)	
Grade III	15 (39.5%)	

In terms of semen parameters, the mean semen volume was significantly lower in Group A than in Group C (2.68±0.99 mL vs. 3.31±1.05 mL, $P=0.0219$); however, mean pH did not show significant differences between study groups, but the mean liquefaction time of semen was slightly higher in Groups A and B than in Group C, without statistical significance (Table 3). We found that sperm concentration, total sperm count, sperm progressive motility, and sperm progressive+non-progressive motility were significantly lower in Group A and Group B than in Group C; however, there were no differences between Group A and Group B regarding these parameters (Table 4). The sperm morphology index was significantly lower in Group A than in Group C ($P=0.0024$); no differences were found between Group B and Group C and Group B and Group A (Table 5). According to the anatomic-morphological characteristics of sperm, Group A was characterized by significantly smaller dimensions of the length and width of the head, its area, and perimeter, as well as the acrosome coverage, compared to both Group C and Group B (Table 6). The mean value of MAI and TZI did not significantly differ between study groups ($P=0.2573$ and $P=0.2480$, respectively). However, the mean value of SDI was significantly lower in Group A than in Group C ($P=0.004$) (Table 7).

Table 3.

Semen analysis (macroscopic examination) in the study groups.

Parameter	Group A (1)	Group B (2)	Group C (3)	Statistics
Volume, mL	2.68±0.99	3.17±0.81	3.31±1.05	F=4.2025 P=0.0177 P_{1-2}=0.0837 P^{1-3}=0.0219 P_{2-3}=0.8303
pH	7.51±0.12	7.52±0.11	7.50± 0.11	F=0.2426 P=0.7851
Liquefaction time, min	27.89±14.73	28.21±11.49	21.73±5.17	F=3.1638 P=0.0466 P_{1-2}=0.9924 P^{1-3}=0.0783 P_{2-3}=0.0715

Table 4.

Semen analysis (microscopic examination) in the study groups.

Parameter	Group A (1)	Group B (2)	Group C (3)	Statistics
Sperm concentration, 10^6/ml	23.63±24.97	20.73±24.02	55.88±22.71	F=20.7786 P=0.0000 P_{1-2}=0.8678 P^{1-3}=0.0000 P_{2-3}=0.0000
Total sperm count, 10^6/ml	62.57±76.51	79.81±110.29	178.79±88.66	F=14.8199 P=0.0000 P_{1-2}=0.7128 P^{1-3}=0.0000 P_{2-3}=0.0001
Progressive motility, %	18.39±18.01	14.36±13.44	52.91±12.63	F=61.7070 P=0.0000 P_{1-2}=0.5041 P^{1-3}=0.0000 P_{2-3}=0.0001
Progressive + non-progressive motility, %	32.18±23.04	26.05±15.57	69.08±13.70	F=50.7048 P=0.0000 P_{1-2}=0.3411 P^{1-3}=0.0000 P_{2-3}=0.0001

Table 5.

The sperm morphology index in the study groups.

Parameter	Group A (1)	Group B (2)	Group C (3)	Statistics
Sperm morphology index, %	23.18±18.63	28.16±13.40	35.93±11.58	F=5.9615 P=0.0036 P_{1-2}=0.3543 P^{1-3}=0.0024 P_{2-3}=0.1095

Table 6.

The anatomic-morphological characteristics of sperm in the study groups.

Parameter	Group A (1)	Group B (2)	Group C (3)	Statistics
Head length, µm	3.60±2.04	4.74±0.23	4.78±0.21	F=9.9490 P=0.0001 P_{1-2}=0.0008 P_{1-3}=0.0007 P_{2-3}=0.9913
Head width, µm	2.27±1.29	2.95±0.15	2.94±0.15	F=8.4143 P=0.0004 P_{1-2}=0.0016 P_{1-3}=0.0026 P_{2-3}=0.9520
Length/ width ratio	1.22±0.69	1.62±0.10	1.63±0.09	F=10.4762 P=0.0001 P_{1-2}=0.0005 P_{1-3}=0.0005 P_{2-3}=0.9968
Head area, μm^2	8.44±4.80	11.00±0.84	11.13±0.70	F=8.9930 P=0.0003 P_{1-2}=0.0016 P_{1-3}=0.0012 P_{2-3}=0.9839
Head perimeter, µm	9.81±5.55	12.87±0.55	12.93±0.44	F=7.5856 P=0.0009 P_{1-2}=0.0009 P_{1-3}=0.0254 P_{2-3}=0.5975
Acrosome coverage, %	29.86±19.43	38.97±8.56	33.72±13.69	F=3.3092 P=0.0407 P_{1-2}=0.0311 P_{1-3}=0.5405 P_{2-3}=0.3464

Table 7.

Sperm morphology indices in the study groups.

	Group A (1)	Group B (2)	Group C (3)	Statistics
MAI	2.01±0.37	2.11±0.33	1.97±0.34	F=1.3764 P=0.2573
TZI	1.06±0.15	1.05±0.14	1.01±0.06	F=1.4142 P=0.2480
SDI	0.73±0.24	0.68±0.20	0.57±0.13	F=5.5087 P=0.0054 P_{1-2}=0.5454 P_{1-3}=0.0040 P_{2-3}=0.0787

Discussion

Varicocele is a common condition found in 15% of the general population and 19%-41% of infertile males,[1-3,21] making it the second most prevalent cause of infertility after idiopathic infertility. Despite the considerable frequency of varicocele in subfertile individuals and proven spermatogenic failure, the specific mechanisms behind varicocele's negative impact on fertility remain unclear.[22] Nevertheless, it affects all sperm characteristics, including count, motility, and morphology.[23]

Sperm morphology, a reflection of intricate cellular changes during spermiogenesis, has been identified by some experts as a particularly robust predictor of fertility.[12,24] This association between sperm morphology and fertility has been established in numerous species, emphasizing the critical role of sperm morphology in fertility assessment.[25] Beyond mere motility, sperm morphology encapsulates vital genetic and DNA characteristics.[26]

Our investigation uncovered a strong link between infertility and sperm morphology across three distinct groups: healthy fertile males, subfertile individuals with varicocele, and those with idiopathic infertility. Healthy fertile males show normal semen characteristics (volume, count, motility, and morphology), as reported by Aziz et al.[27] and Ahmad et al.[28] Based on semen characteristics and the existence of a varicocele, subfertile individuals were divided into groups, as previously investigated by Pasqualotto et al.[29] and Blumer et al.[30]

We found that sperm concentration, total sperm count, sperm progressive motility, and sperm progressive+non-progressive motility were significantly lower in patients with varicocele and idiopathic infertile males than in healthy fertile controls (P=0.000 in all cases). This aligns with findings reported by Vivas-Acevedo et al.,[31] highlighting decreased sperm motility in infertile males with varicoceles. However, it is worth noting that Saleh and Agarwal[32] found no substantial disparities in sperm motility between infertile males and fertile controls.

Furthermore, our study revealed that the mean sperm morphology index was significantly lower in subfertile patients with varicocele than in healthy fertile men (P=0.0024). These observations align with the results of Tawadrous et al.,[33] Mostafa et al.,[34] and Vivas-Acevedo,[35] who reported similar findings.

Conversely, the WHO study indicated that infertile males with varicocele exhibited reduced sperm concentration but did not provide specific evidence concerning motility and morphology.[36] Some researchers postulate that the observed low sperm concentration may be attributed to the elevated rate of germ apoptosis often found in men. In contrast, diminished motility may be linked to a high concentration of reactive oxygen species or anti-sperm antibodies.[37,38]

Semen analysis normally evaluates only the dimensions of the sperm head (WHO, 1999)[12] because head morphological anomalies significantly affect male fertility.[39] However, despite WHO recommendations to consider additional aspects of sperm morphology, little attention has been given to the diameters of the midpiece and flagellum.[40]

Our study revealed significant deviations in head lengths, perimeters, and acrosome coverage in patients of the studied groups. Subfertile patients with varicocele were characterized by significantly smaller dimensions of the length and width of the head, its area, and perimeter, as well as the acrosome coverage than in fertile men.

These findings echo the results of Vazquez Levin[41] and Schatte,[42] who identified a lower frequency of morphologically normal forms in varicocele patients when stringent criteria were applied. In contrast, Saleh and Agarwal[32] observed no significant differences in sperm morphology between infertile individuals and fertile controls. MacLeod in 1965[43] identified the "stress pattern," characterized by elongated tapering sperm heads and amorphous spermatozoa linked with varicocele.

However, Rodrigues-Rigau et al.[44] found no notable changes in sperm shape between males with and without varicocele. WHO (1999) also observed a substantial negative correlation between average head length and the proportion of sperm with "normal" morphology.[12]

Our study also revealed a significantly reduced sperm deformity index in subfertile patients with varicocele, compared to fertile men, suggesting that increased abnormality in head length and perimeters is one of the possible causes of infertility due to varicocele. Wang et al.[45] observed that a 1°C increase in testicular temperature inhibits spermatogenesis by 14%, resulting in a drop in sperm production. Additionally, exposure to extreme temperatures alters the shape of sperm, resulting in a rise in sperm with aberrant morphology. Within 6-8 months of exposure to high temperatures, the average percentage of sperm with aberrant morphology increases from 30% to 60%. The researchers hypothesized that heating the testes decreased the quantity and the quality of sperm production.[45] Activation of the *p53* gene, a tumor-suppressor gene expressed in testes, is a well-known mechanism for explaining spermatogenic dysfunction caused by heat.[46,47] It is most highly expressed in pachytene spermatocytes.[48] High scrotal temperatures result in condensation of nuclear chromatin, which activates *p53* and halts the cell cycle. This hinders the clonal expansion of germ cells with DNA damage. Morgentaler et al.[49] hypothesized that *p53* may be involved in heat-induced germ-cell death. *p53* is situated on the nuclear membrane of normal germ cells and is responsible for germ-cell quality control. With heat-induced nuclear damage, it translocates to the nucleoplasm and triggers germ-cell death.[50]

In conclusion, our study highlights the significant association between sperm morphology and male infertility in varicocele and idiopathic subfertile males. Further research is needed to explore the relationship between sperm morphometry, sperm function, and fertility across different species. Additionally, understanding the therapeutic implications of sperm morphology could aid in selecting semen samples with the least aberrant morphometry for subfertile men.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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