

# Sperm Concentration, Motility, and Morphology in Subfertile Men

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## ABSTRACT

Infertility is a growing global health concern that affects more than 70 million couples annually. Fifty percent of cases of infertility are caused by male factors, which are mostly caused by deficiencies in sperm quantity and/or quality. This study aimed to analyze sperm morphology, motility, and concentration in subfertile men to understand potential factors contributing to subfertility. A case-control study involving 150 male participants, including 50 fertile men and 100 subfertile men, was conducted. Semen samples were collected and analyzed according to WHO guidelines. Subfertile men showed a significantly lower semen concentration, count, and motility, as well as a lower rate of normal morphological sperm compared to fertile men. There was also a significant association between subfertility and smoking. However, no statistically significant differences were observed in sperm head abnormalities between subfertile and fertile men. These findings suggest that sperm morphology may not be a reliable indicator of subfertility in all cases. Subfertile individuals typically display lower semen parameters. Smoking was identified as a potential contributing factor to male subfertility.

**Keywords:** Subfertile, Male factor infertility, Semen analysis, Sperm morphology, Sperm motility.

## INTRODUCTION

Infertility is a reproductive health issue affecting millions of couples globally, with approximately 15% of couples experiencing difficulties conceiving, it is defined as the failure to conceive after at least 12 months of consistent, unprotected sexual activity in a fertile female. However, infertility is often depicted as primarily a female issue, male infertility factors play a significant role, accounting for nearly 50% of infertility cases (Evans et al., 2021).

Male infertility has been linked to a number of conditions, including sperm DNA fragmentation, spermatogenesis abnormalities, hormone imbalances, and genetic diseases. Among these factors, abnormalities in sperm morphology, motility, and concentration play crucial roles in determining male fertility potential (Kamiński et al., 2020).

Mature sperm exhibits a unique structure optimized for fertilization and motility. Normal sperm typically exhibits an oval head shape that contains a distinct acrosome, below the head is the mid-piece, which is slightly thicker than the tail. A single tail 50 µm in length should be attached to the head by the mid-piece. Abnormal sperm shows different morphological abnormalities including head defects (large, small-tapering, duplicate, or amorphous), mid-piece defects, and tail defects (double or coiled) (Patel, 2023). Sperm morphology plays a crucial role in fertility outcomes. Abnormal sperm development can hinder the fertilization process, leading to infertility. Studies have shown that poor sperm morphology is associated with lower fertilization, cleavage, and pregnancy rates, highlighting the importance of good sperm morphology for successful outcomes in assisted reproductive technologies (Yaramareddy et al., 2023).

According to WHO criteria, ABCD score is a system used to assess sperm motility in male fertility, sperm motility was categorized into rapid progressive (grade a), slow progressive (grade b), non-progressive (grade c), and immotile (grade d) (Haugen et al., 2023). Sperm motility plays a significant role in male fertility as it allows sperm to travel through the female reproductive tract and fertilize an egg and is directly linked to fertilization success in both natural and assisted reproduction (Gai et al., 2022). Studies have shown that high-speed and straight-lined motion of sperm correlate positively with fertility, suggesting their importance in reproductive success (Fernández-López et al., 2022).

A normal sperm count is typically considered to be more than 15 million sperm per milliliter of semen according to World Health Organization (WHO) criteria. A lower sperm count may indicate potential fertility issues. Some studies suggest that higher sperm concentrations can lead to higher fertilization rates (Zacà et al., 2020), while other studies indicate that sperm concentration is not a consistent predictor of fertility, and factors such as sperm chromatin condensation and motility may be more relevant (Aghazarian et al., 2020). The objective of this study is to analyze sperm morphology, motility, and concentration in subfertile men to understand potential factors contributing to subfertility and to aid in the development of targeted interventions or treatments.

## MATERIAL AND METHODS

This case-control study included 150 Male; 50 as healthy fertile men who achieved a pregnancy within the last year, and 100 subfertile men, aged between 20 – 65 years, patients were collected from those attending to infertility clinic in Ar-Razzi IVF hospital in western Iraq during the period from October 2022 to October 2023. The patients had at least one semen parameter below the WHO-recommended reference value. Semen collection and analysis followed the guidelines outlined in the WHO guidelines for the examination of human semen, 6th edition (Baldi et al., 2022). Semen samples were collected in the laboratory following two to five days of not having sex to ensure optimal sperm quality in a sterile sperm collection container labeled with participant ID and collection date. The semen samples were mixed gently to ensure homogeneity and left to liquefy at a temperature of 37°C for 20-30 minutes. Sperm morphology analysis carried out by spreading a 10 µl of well-mixed semen onto a clean microscope slide stained with Diff-quick kit, sperm morphology has been examined under a microscope at high magnification (1000x) according to established criteria, observing abnormalities such as head defects, tail defects. Sperm motility was recorded and categorized into progressive motility, non-progressive motility, and immotility. The Sperm Count was carried out with the counting chamber's hemocytometer.

### Inclusion criteria

The study included male participants of a 1-year history of male subfertility. patients had abnormal semen analysis at least one semen parameter below the reference value recommended by WHO (2010).

### Exclusion criteria

Azoospermic patients, individuals with family history of infertility, who have chronic illnesses which have an impact on their fertility, or abnormalities related to anatomy, hormones, and genetics, history of testicular trauma or treatment for vasectomy, or had received antibiotics or surgery in the month prior to the study were excluded from the study.

### Ethical Approval

This study was approved by the by the local ethical committee Iraqi Ministry of Health. All participants had given informed consent to participate in this study.

### Statistical Analysis

The SPSS software, version 26, was utilized for statistical analysis (SPSS Inc., Chicago, IL, USA). The expression for continuous variables was mean  $\pm$  standard deviation (SD). Frequencies and percentages are used to represent categorical variables. Continuous data were compared using the student t test. Chi squared  $\chi^2$  was used for evaluate the association between categorical variables (Borg et al., 2023).

## RESULTS

A comparison between the studied groups revealed a non-statistically significant difference in age ( $p = 0.793$ ) or BMI ( $p = 0.519$ ). However, subfertile men exhibited significantly higher rates of smoking ( $p = 0.02$ ) as shown in Table 1.

**Table 1:** characteristics distribution of subfertile and fertile men.

		Fertile men	Subfertile men
Age	Mean $\pm$ SD	33.3 $\pm$ 5.5	33.6 $\pm$ 7.5
	P value	0.793	
BMI	Mean $\pm$ SD	27.4 $\pm$ 4.7	26.9 $\pm$ 4.9
	P value	0.519	
Smoking (%)	Yes %	30 %	56 %
	No %	70 %	44 %
	P value	0.02*	

Table 2 demonstrates that the subfertile men group exhibited significantly lower semen concentration (p-value < 0.0001), sperm count (p-value < 0.0001), and motility (p-value < 0.0001), as well as a lower percentage of normal morphological sperm (p-value < 0.0001) compared to the fertile group.

**Table 2:** Comparison between fertile and subfertile men groups regarding the semen parameters

		Fertile men	Subfertile men
	Mean± SD	83.6 ± 20.0	56.5 ± 33.3
Concentration	P value	0.0001*	
Total count (M)	Mean± SD	281.6 ± 95.0	153.7 ± 105.7
	P value	0.0001*	
Total motility (%)	Mean± SD	67.3 ± 10.1	41.9 ± 24.7
	P value	0.0001*	
Total morphology (%)	Normal	96.2±0.25	1.14± 0.11
	Abnormal	3.8±0.25	98.8±0.11
	P value	0.0001*	

As shown in Table 3, there is a statistically significant difference between Subfertile and fertile men regarding the type of sperm motility, our results have demonstrated a significant association between subfertile men with lower rates of rapid progressive, slow progressive, and non-progressive, as well higher rates of immotile sperm compared to fertile men.

**Table 3:** Comparison between the studied groups regarding the type of motility

Motility	Fertile men	Subfertile men	$\chi^2$	P value
	Mean± SD	Mean± SD		
A (%)	3.7±0.78	0.2±0.09	16.3	0.001*
(%)B	9.8±0.88	2.5±0.44		
C (%)	53.8±11.5	38.5±2.33		
D (%)	33.0±1.7	57.6±2.45		

Unexpectedly, upon comparison between the studied groups, no statistically significant differences were observed in sperm morphology. subfertile men exhibited similar sperm head abnormalities compared to fertile men (p = 0.899).

**Table 4:** Comparison between the studied groups regarding the sperm head abnormalities.

Morphology	Fertile men	Subfertile men	$\chi^2$	P value
	Mean± SD	Mean± SD		
Small head (%)	10.8±2.9	10.7±7.1	2.206	0.899
(%)Round head	13.2±4.4	10.4±4.7		
Large head (%)	10.0±3.6	10.2±6.2		
Duplicate head (%)	0.5±1.35	1.08±2.68		
Tapered head (%)	14.2±3.73	12.7±5.54		
Amorphous head (%)	11.2±3.7	15.5±6.3		
Pin head (%)	10.9±3.2	9.3±5.0		

## DISCUSSION

Subfertility represents a significant public health issue, with implications for individuals and societies worldwide. Beyond the lives of couples, subfertility also impacts the social life of individuals and their communities. Male factor subfertility accounts for 20%–50% of infertile couples. (Pourabbas et al., 2023). Male infertility can be attributed to various reasons, including pre-testicular, testicular, and post-testicular ones (Craciunas & McEleny, 2024). However, leukocytospermia, DNA Fragmentation Index (DFI), is linked to factors like smoking, alcohol consumption and reactive oxygen species (ROS) could also affect fertility status (Malhotra, Gouri Devi & Patil, 2024). Male infertility can be treated in a variety of ways, depending on the underlying cause. Assisted reproductive technologies are a major factor in the management of male fertility. Lifestyle changes, medication, and surgery interventions are additional treatment options (Craciunas & McEleny, 2024).

A semen analysis is typically the primary diagnostic test for male infertility. Semen abnormalities have been shown to be a major cause of infertility. Semen analysis involves evaluating various parameters such as

morphology, concentration, count, and volume. Additionally, motility, especially progressive motility, and vitality are also assessed. A subfertile male always means an abnormal or subnormal semen analysis. Poor semen quality may be characterized with abnormal semen parameters include decreased ejaculate volume, semen count and motility, higher sperm morphology abnormalities, and extended sperm liquefaction time (Huang et al., 2023). Semen parameters could be influenced by various factors such as sexual abstinence periods, abnormal hormonal levels, testicle, body mass index (BMI), infections and antibiotics intake, diet regimen, and lifestyle (Milachich & Dyulgerova-Nikolova, 2020).

The aim of this study is to analyze sperm morphology, motility, and concentration in subfertile men to understand potential factors contributing to subfertility.

From our results, Age and BMI were matched in the studied groups. However, there is a significant association between subfertility and smoking. These findings align with prior researches that have established a positive correlation between increased smoking and male subfertility, oligospermia, and morphological defects were significantly higher among smokers compared with non-smokers (Bundhun et al., 2019). Additionally, smokers have a decreased sperm motility, count, and shape (Sharma et al., 2016). The mechanism behind this association can be illustrated through the role of smoking in inducing oxidative stress which impairs sperm quality (Mostafa et al., 2018). In another mechanism, smoking is related to lower levels of zinc, the responsible for the process of spermatogenesis and its deficiency may halt the process and additionally impacts sperm motility and viability (Bazid et al., 2022).

Our results demonstrated that subfertile men exhibited significantly lower semen concentration and count, reduced motility, and a decreased percentage of normal morphological sperm compared to fertile men. These findings are supported by numerous reports that have shown a difference in the main semen characteristics between the fertile and subfertile groups (M et al., 2022). Recent studies coincide with our findings, demonstrating an association between subfertile men and lower sperm concentration, and motility as well as poor sperm morphology in comparison with fertile men, the author suggested this dramatic deterioration in semen quality would result from urogenital tract infection (Eini et al., 2021). Furthermore, sperm concentration and motility have significantly decreased among subfertile men associated with more frequent morphological abnormalities compared to fertile men according to a Saudi study (Aleisa, 2013).

In our study, we observed an association between subfertile men with lower rates of rapid progressive, slow progressive, and non-progressive, as well as higher rates of immotile sperm compared to fertile men. Several studies have supported our finding, revealing that the progressive motility was the best parameter with sperm morphology to differentiate subfertile from fertile men. A Turkey study investigated the semen parameters in fertile men in comparison with men subfertile. It showed that subfertile men exhibit lower rates of rapid progressive compared to fertile men (Gunalp et al., 2001). Also in the same line, another Iraqi study has confirmed our findings. The result of this study showed that subfertile men exhibit lower rates of rapid progressive motility and higher rates of non-motility compared to fertile men (Al-Ali & Hashim, 2019).

However, several studies have highlighted that the morphology of spermatozoa including the size, shape and appearance is an important predictive factor for outcome of natural conception. Surprisingly, in our population the result of this study failed to find a statistically significant difference in sperm head abnormalities between subfertile and fertile men. In line with our results, a study conducted in France to investigate the potential diagnostic value of sperm head abnormalities in subfertile men, it failed to distinguish subfertile and fertile men based on head abnormalities, they did not find any significant difference (Gatimel et al., 2014). Furthermore, Moreover, numerous reports interested in investigating the morphological abnormalities of the spermatozoa that may contribute to infertility, these reports showed that there were no substantial differences in sperm abnormalities were found between subfertile and fertile (Panidis et al., 2003; Kubo-Irie et al., 2005). This suggests that sperm morphology may not be a primary cause of infertility, unlike sperm concentration and motility, which are often more indicative of fertility status (Gatimel et al., 2017; Kohn, Kohn & Lamb, 2018).

## CONCLUSION

The results of the study demonstrate that semen analysis has the potential to distinguish subfertile men. Subfertile individuals typically display lower semen concentration, sperm count, and motility, as well as a higher incidence of abnormal sperm morphology compared to fertile men. Interestingly, our results revealed no statistically significant differences in sperm head abnormalities between subfertile and fertile men, suggesting that sperm morphology may not be a reliable indicator of subfertility in all cases. Additionally, smoking emerged as a significant factor associated with subfertility in this study.

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