



# Significance of Sperm Characteristics in the Evaluation of Male Infertility in a Tertiary Care Centre

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## ABSTRACT

**Background:** Infertility is both a clinical and a public problem. Standard semen analysis is the surrogate measure of male fertility in clinical practice to determine prevalence of low sperm count including oligozoospermia and azoospermia and to assess the pattern and distribution of abnormal semen parameters in infertile men.

**Methods:** The retrospective study was conducted with compiling of the data from archival record over a period of three years from June 2013 to June 2016. A total of 933 male partners of women attending the fertility clinic of hospital between the ages of 20 and 50 years were recruited. The samples taken were primary infertility cases using simple random sampling technique. Semen analysis was performed according to the standards outlined by the World Health Organization (5th edition 2010). Parameters outlined included: Appearance, Volume, pH, Sperm concentration, Motility, Morphology, Viability and White cell count.

**Result:** Out of 933 samples, normozoospermia was observed in 659 (70.6%) males, oligozoospermia 170 (18.2%), and azoospermia 104 (11.1%). The azoospermic and oligozoospermic samples had low ejaculated volume, but significantly higher percentage of pus cells in comparison to normozoospermic samples. The oligozoospermic samples had higher percentage of immotile sperm and abnormal morphology in comparison to normozoospermic samples. Asthenozoospermia was observed in 118 (14.2%), teratozoospermia in 24 (2.9%), and oligoteratozoospermia in 11 (1.3%) of samples.

**Conclusion:** Majority of cases of infertility in males show normal sperm count. Oligozoospermia followed by azoospermia is seen in rest of the cases while less sperm motility or less amount of semen are also responsible in some cases.

**Keywords:** Semen Analysis, Male Infertility, Sperm Motility, Morphology, Azoospermia.

## Introduction

Infertility is a comprehensive issue affecting approximately 13-15% of the population all over the world.<sup>[1]</sup> A host of factors have been implicated in the causation of infertility.<sup>[1]</sup> The female factor is responsible for 35% of cases whereas the male factor is seen in 45% of cases. The remaining 20% of the couples either have non-identified infertility or mixture of factors.<sup>[2]</sup>

The role of semen analysis in the assessment of male fertility is paramount and remains the most fundamental and primary investigation.<sup>[3]</sup> The use of standardized and objective procedures ensures satisfactory categorization of cases of infertility.<sup>[3]</sup> The results of the test provide vital information regarding the concentration, motility and morphology of the spermatozoa in a semen sample.<sup>[1]</sup> The sensitivity value of standard semen analysis is 89.6%, which implies that it has the ability to identify 9 out of 10 men having a genuine problem.<sup>[4]</sup> Semen analysis allows for a better understanding of the structural and dynamical parameters involved in sperm function.<sup>[2]</sup> The analysis also helps to elucidate the pathological causes for decreased

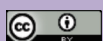
sperm count thereby classifying the issue into a pre-testicular, testicular or post testicular phase.<sup>[5]</sup>

The majority of male infertility (90%) is due to low sperm number, poor semen quality or a combination of both. Worldwide collected data suggest that there has been a continuous decline in semen quality and quantity.<sup>[6]</sup> This alarming finding can be attributed to increased prevalence of sexually transmitted diseases (STDs), urogenital infections and modern lifestyle influences.<sup>[7]</sup> In our population where infertility is considered to be a social stigma and the female is often held responsible for the inability to conceive, screening by semen analysis to rule out the male factor is imperative before subjecting the couple to extensive investigations.<sup>[8]</sup>

In this study we determine the frequency of low sperm count in Indian population and semen parameters include sperm motility and morphological details to identify abnormalities in semen.

## Materials and Methods

The retrospective study was carried out in the Department of Pathology, Government Medical College and Hospital,



Chandigarh, from June 2013 to June 2016. All these couples were unable to conceive for at least 12 months. All the cases, which included the study, were archived from hospital records.

933 Samples of male partners (933) of women attending the fertility clinic of the hospital between the ages of 20 and 50 years were evaluated. The samples taken were primary infertility cases. Cases of secondary infertility were excluded from the study.

Analysis of semen was performed according to the standard methods outlined by the World Health Organization (WHO laboratory manual for the examination and processing of human semen 5<sup>th</sup> edition 2010). Parameters outlined included: Appearance: grey/opalescent; Volume: 2.0ml or more; pH: alkaline i.e. 7.2-7.8; Sperm concentration:  $>15 \times 10^6$  spermatozoa/ml; Total sperm count:  $39 \times 10^6$  per ejaculate or more; Motility: 40% or more including progressive and non progressive motility; Morphology: 4% or more with normal forms; Viability: 58% live spermatozoa; White cell count:  $<1 \times 10^9$ /ml.

Complete sample collection and analysis was done by the same lab technician to avoid inter-laboratory variation. Within 60 minutes of collection, semen analysis was performed and parameters included appearance, morphology, motility, volume, liquefaction, pH, concentration, viability and the occurrence of pus cells. Disposable pipette (graduated) were used to measure semen volume; pH test was done with the help of pH paper. After liquefaction, sperm motility was assessed by microscopic evaluation of 200 spermatozoa from different fields. Counting of spermatozoa was done using improved Neubauer's chamber. Viability was assessed with eosin stain. The semen samples were categorized on the basis of sperm count/mL of semen in accordance with WHO normal and pathological ranges i.e. normozoospermia (normal sperm count), oligozoospermia, (total number (or concentration, depending on outcome reported) of spermatozoa below the lower reference limit) and azoospermia (no spermatozoa in the ejaculate). The different samples were categorized and compared for ejaculated volume, sperm count, viability, pus cells, motility and morphology.

The following operational definitions were used: Normozoospermia: Sperm count 15 million/ml to 150 million/ml; Oligozoospermia: Sperm count below 15 million/ml; Azoospermia: Complete absence of spermatozoa in the ejaculation; Asthenozoospermia: Reduced sperm motility below the lower reference limit; Teratozoospermia: Abnormal sperm morphology;

Oligoasthenoteratozoospermia: All sperm variables abnormal; Hypospermia: Volume  $<2$ ml; Normospermia: Volume 2-5 ml; and Hyperspermia: Volume  $>5$ ml.

The data was analysed using SPSS software (version 15). Mean  $\pm$  Standard deviation (SD) were calculated for sperm count, volume, motility, morphology and pus cells; 95% confidence interval was calculated for proportions and for means. Mean values were also compared for statistical significance using t-value with level of significance  $<0.05$  (p value).

## Result

A total 933 semen analysis reports of male partners of infertile couples were analyzed over a period of 3 years. Among the 933 males, the mean age was  $30.02 \pm 4.72$  years. Using WHO standard for semen normality, 933 semen samples were analysed, out of these 659 (70.6%) had normozoospermia, 170 (18.2%) had oligozoospermia and 104 (11.1%) azoospermia, as depicted in Table 1. On the basis of semen volume, samples were categorized as normospermia (2-5ml), hypospermia ( $<2$ ml), hyperspermia ( $>5$ ml). The distribution of semen volume is shown in Table 2.

After excluding 104 samples with azoospermia, semen parameters were compared in oligozoospermic and normozoospermic samples for count/sperm concentration (million/ml), volume (ml), liquefaction time (min), viability (%), motile sperms (including progressive motile and non-progressive motile sperms), immotile sperms, morphologically normal sperms and abnormal sperms (including head, neck and tail abnormalities) and pus cell (per HPF). The oligozoospermic samples had significantly higher percentage of immotile sperms  $55.79 \pm 28.00$  and abnormal morphology  $23.64 \pm 27.28$  compared to normozoospermia in which non-motile sperms were  $33.32 \pm 19.40$ , and abnormal morphology was  $11.45 \pm 9.74$  respectively (p  $<0.001$ ).

Comparison of volume showed mean volume of  $2.64 \pm 1.38$ ml in normozoospermia vs  $2.25 \pm 1.17$ ml in oligozoospermia (p 0.002), and pus cells  $4.33 \pm 4.38$ /HPF in normozoospermia vs  $5.71 \pm 6.56$ /HPF in oligozoospermia. This was statistically significant (p 0.009) (Table 3). Normal motility was observed in  $66.66 \pm 19.99$  of normozoospermic vs  $44.38 \pm 28.20$  of oligozoospermic samples, and normal morphology of sperms was observed in  $88.54 \pm 9.74$  of normozoospermic vs  $76.43 \pm 27.27$  of oligozoospermic samples (p  $<0.001$ ).

Comparison of sperm viability showed mean viability of  $69.87 \pm 26.69$  in normozoospermia vs  $51.78 \pm 24.15$  in oligozoospermia (p  $<0.001$ ) and liquefaction time

42.76±10.20 min in normozoospermia vs 44.25±10.67min in oligozoospermia. This was not statistically significant (p 0.093) (Table 3).

The proportion of multiple factor abnormalities defects were seen in 253 cases out of 829 cases of both normozoospermia and oligozoospermia as given in Table 4.

**Table 1: Frequency of sperm concentration**

| Category        | Frequency (N=933) | Percentage (%) |
|-----------------|-------------------|----------------|
| Normozoospermia | 659               | 70.6           |
| Oligozoospermia | 170               | 18.2           |
| Azoospermia     | 104               | 11.1           |

**Table 2: Distribution of semen volume**

| Volume               | Frequency (N=933) | Percentage (%) |
|----------------------|-------------------|----------------|
| Normospermia (2-5ml) | 658               | 70.5           |
| Hypospermia (<2ml)   | 252               | 27.0           |
| Hyperspermia (>5ml)  | 23                | 2.4            |

**Table 3: Comparison of semen parameters between normozoo-spermia and oligozoo-spermia**

| Category                | Count mean±SD | Volume mean±SD | Viability mean±SD | Pus cells mean±SD median±IR | Motile sperm mean±SD | Immotile sperm mean±SD | Normal sperm mean±SD | Abnormal sperm mean±SD |
|-------------------------|---------------|----------------|-------------------|-----------------------------|----------------------|------------------------|----------------------|------------------------|
| <b>Normozoo-spermia</b> | 84.98±32.87   | 2.64±1.38      | 69.87±26.69       | 4.33±4.38                   | 66.66±19.99          | 33.32±19.40            | 88.54±9.74           | 11.45±9.74             |
| <b>95%CI</b>            | 82.46-87.49   | 2.53±2.74      | 67.83-71.91       | 4.00-4.67                   | 65.13-68.19          | 31.84±34.80            | 87.79-89.28          | 10.71±12.20            |
| <b>Oligozoo-spermia</b> | 9.78±3.82     | 2.25±1.17      | 51.78±24.15       | 5.71±6.56                   | 44.38±28.20          | 55.79±28.00            | 76.43±27.27          | 23.64±27.28            |
| <b>95%CI</b>            | 9.20-10.36    | 2.08±2.43      | 48.12-55.44       | 4.72-6.71                   | 40.11-48.65          | 51.55±60.03            | 72.29-80.55          | 19.50±27.77            |
| <b>P value</b>          | <0.001        | 0.002          | <0.001            | 0.009                       | <0.001               | <0.001                 | <0.001               | <0.001                 |

**Table 4: Proportion of multiple factor abnormalities defect**

| Pattern of abnormalities     | Frequency | Percentage (%) |
|------------------------------|-----------|----------------|
| Asthenozoospermia            | 118       | 14.2           |
| Teratozoospermia             | 24        | 2.9            |
| Asthenoteratozoospermia      | 13        | 1.39           |
| Oligoasthenozoospermia       | 68        | 8.2            |
| Oligoteratozoospermia        | 19        | 2.3            |
| Oligoasthenoteratozoospermia | 11        | 1.3            |

## Discussion

Infertility has long been a subject of debate and the females have always had to bear the brunt of the socio-cultural connotations of this multifaceted issue.<sup>[1,10]</sup> Advancements and progress of novel assisted reproductive techniques establish males to be an equal, if not higher contributor to this complex problem.<sup>[2]</sup> Despite education and enlightenment, the social attitude towards infertility results in much trauma, emotional instability and psychological stress, which in turn has an adverse bearing

on the physiology and psychology of the individual, particularly in a social set-up such as ours, where there has been a strong emphasis on child-bearing.<sup>[11]</sup> Semen analysis provides some insight about the pathology of epidemiological problems occurring in the male genital tract.<sup>[1,4]</sup> As high as majority (90%) of male infertility problems are connected to sperm count and a positive association between abnormal semen parameters and sperm number, has been observed. The problem of sperm count, motility and morphology stems from disarray in

control mechanisms, including pre-testicular, testicular and post-testicular factors.<sup>[12]</sup>

In our study it was found that of total 933 cases 659 (70.6%) males had normal sperm count and rest 274 (29.3%) males had abnormal semen analysis report. This is similar to a study done in 2012 which reported the incidence of male infertility as 62%.<sup>[7]</sup> The reported prevalence of oligozoospermic, azoospermic, asthenozoospermic and asthenoteratozoospermic in cases of primary infertility in same study was 33.17%, 9.89%, 1.83 and 1.08% respectively,<sup>[13]</sup> which were similar to our study results. The prevalence of azoospermia in our study population was 10.70%, oligozoospermia 34.14%, asthenozoospermia 14.2%, and of asthenoteratozoospermia 1.39% respectively.<sup>[7]</sup> The results are comparable to study which reported the occurrence of azoospermia as 14.28% and that of oligozoospermia 21.43%,<sup>[14]</sup> in another study, the incidence rate of azoospermia was 16%.<sup>[15]</sup>

Mean ejaculated volume in normozoospermia was  $2.64 \pm 1.38$  ml vs  $2.25 \pm 1.17$  ml in oligozoospermia and  $2.20 \pm 1.30$  ml in azoospermic samples respectively. Majority of our patients had normal semen volume 70.5%, while 27.0% showed hypospermia (<2ml), and hyperspermia in 2.4%, these results can be comparable to a study conducted in Sudan where majority of the subjects (89.7%) had adequate semen volume, while only 10.3% had abnormal semen volume.<sup>[16]</sup> Moreover, these results are also analogous to a study conducted in Nigeria in which majority of the subjects (91%) had adequate semen volume, while only 9% had abnormal semen volume i.e 7.3% hypospermia and 1.7% hyperspermia.<sup>[17]</sup> The adequate semen volume obtained in our study may be a result of the 3-6 days of sexual abstinence.

In normozoospermia samples, the mean percentage of normal motile sperms was  $57\% \pm 0.18$  as compared to oligozoospermia in which motile sperms were  $38\% \pm 23\%$ . However, advancing techniques to some extent overcome the problems of sperm motility in infertile couples, but asthenozoospermia is still a common cause of human male infertility. In our study, asthenozoospermia was observed in 14.2% of samples and the results were comparable to a study conducted at the National Institute of Health, Islamabad, in which the prevalence was around 21.42%.<sup>[18]</sup> In another study, the prevalence of asthenozoospermia was 18%.<sup>[19]</sup>

Morphology of a sperm i.e. the differential development of the head, mid-piece and tail is a function of testes as well as the epididymis. In this study in normozoospermia samples, mean normal morphology was found to be  $88.54 \pm 9.74\%$

vs.  $76.43 \pm 27.27\%$  in oligozoospermic samples. The oligozoospermic samples had significantly higher abnormal motility  $55.79 \pm 28.00\%$  and abnormal morphology  $23.64 \pm 27.28\%$  as compared to normozoospermic samples with  $33.32 \pm 19.40\%$  abnormal motility and  $11.45 \pm 9.74\%$  abnormal morphology. These results are comparable to a study in which abnormal morphology was observed in 53% and abnormal motility in 60% oligozoospermic males.<sup>[20]</sup> So sperm motility and morphology are changing parameters and their relative levels depend on the existing sperm count in an individual.<sup>[20]</sup>

Oligozoospermic samples were found to be associated with significant higher abnormal motility  $62\% \pm 0.239$  and abnormal morphology  $55\% \pm 0.156$  as compared to normozoospermic samples although we did not specify the type of abnormal morphology. The results are comparable to a study in which abnormal morphology was observed in 53% and abnormal motility in 60% oligozoospermic males. So sperm motility and morphology are changing parameters and their relative levels depend on the existing sperm count in an individual.<sup>[20]</sup>

The prognosis of the infertile couple is inversely proportional to the number of abnormal patterns so one pattern of abnormality is better than two-pattern abnormality, and two is better than three-factor abnormality.<sup>[21,22]</sup> When three-pattern abnormalities were identified in oligozoospermic sample population, the prevalence of oligoasthenoteratozoospermia was 1.3%. The results were comparable to a study in which prevalence of oligoasthenoteratozoospermia was 11%.<sup>[18]</sup> The prevalence of teratozoospermia in our study population was 2.9%.

## Conclusion

Semen analysis is primary tool to investigate male infertility which is more useful in developing countries like India. It comes under basic investigation done at minimal rates for infertility cases. It is a cost effective, more reproducible and gives robust information of male reproductive function. The use of conventional parameters, such as sperm count, viability, sperm morphology and motility are markers of male reproductive function. Thus, semen analysis serves as a preliminary investigation to rule out male cause of infertility.

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## Competing Interests

There are no conflicts of interest.

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