

## Original Article

# Recommendation to Diagnose Male Factor Infertility Based on Routine Semen Analysis Results

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## Abstract

It is apparent that sperm parameters like concentration, motility and morphology are not definitive/adequate fertility measures as sperm is a heterogeneous mixture, and sperm quality is determined by only what is visible in the microscopic field. Objective was to assess the quality variation among different aliquots of an ejaculate and present a concept that may allow interpreting the results in such a way as to determine the clinical usefulness of the semen analysis more accurately.

A laboratory-based cross-sectional study was conducted at Teaching Hospital Jaffna's Semiology laboratory from July to September 2023 after obtaining Institutional Ethical Review committee approval, analysing 102 semen ejaculates collected through masturbation. The sample was analysed using a Makler counting chamber, and Data was analysed using SPSS version 24, with statistical significance set at  $P < 0.05$ .

The three different aliquots obtained from the same ejaculate revealed no significant difference from each other for all three variables like Concentration ( $P=0.957$ ), Progressive Motility ( $P=0.810$ ) and Motility ( $P=0.832$ ). Similarly, the mean and fifth percentile plus 95% confidence level were not significantly different ( $P= 0.782$ ,  $P= 0.328$ ,  $P= 0.370$ ).

When using the Makler counting chamber, the mean value calculated from one aliquot per ejaculate counted on three strips of ten squares should be acceptable, and at least two different ejaculates should be analysed when interpreting the results based on World Health Organization (WHO, 2021) reference limits.

## Keywords

Male Factor infertility, Semen analysis, Makler counting chamber.

## Introduction

Subfertility is one of the major health challenges globally and roughly affects 1 in 6 people worldwide. It can be due to male factors, female factors, or a combination of male and female factors, or it may be unexplained (1).

Abnormal sperm parameters frequently describe male infertility; however, it can exist even when the sperm analysis is normal. Sperm concentration, motility, and morphology are clearly not definite or appropriate fertility markers. This is because a considerable change in sperm quality has been documented between consecutive samples collected from the same individual acquired only a few days or even a few hours apart. A significant variation in sperm quality reported between ejaculates taken from the same individual is due to diverse biological, behavioural, and environmental factors that influence semen quality during ejaculation, as detailed in the World Health Organization (2).

Laboratory investigation results are not always adequate for physicians to interpret and manage the patient (3). Ejaculate, like blood, is a heterogeneous mixture of cells and plasma, but unlike blood, sperm quality varies from ejaculate to ejaculate in the same individual. Blood, unlike semen, has a fixed volume of approximately five litres in a human, and a minute sample is electronically analysed by counting thousands of cells. Thus, the result is consistent and repeatable, whereas the sperm quality is determined only by what is visible in the microscopic field. Therefore, basing a diagnosis on a mean value which does not truly represent the variation in an ejaculate appears inadequate.

Semen analysis is an essential technique for predicting conception, correcting medical problems that cause

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lower fecundity, and perhaps resolving long-term health difficulties in subfertile men. Andrologists are now split in their view and practice about the appropriate method and equipment for testing such as morphology. This split results in field malfunction and a lack of structure (4). To improve, a uniform starting point must be followed, allowing evidence-based test modifications for clinical usefulness and quality improvement. The aim of this study was to examine the quality variation across different aliquots of an ejaculate and propose a concept that may allow interpreting the data in such a manner that the therapeutic value of the semen analysis could be determined more precisely.

## Methods

This study was conducted as a laboratory-based cross-sectional study from July to September 2023 at the Andrology laboratory, University unit, Teaching Hospital Jaffna.

This study recruited leftover semen ejaculates obtained by self-masturbation from one hundred two patients who underwent seminal fluid evaluation for sub-fertility after obtaining the patient's informed written consent. Patients who ejaculate with azoospermia were excluded from this study.

Semen ejaculates were analysed using a Makler counting chamber following the manufacturer's recommendation (Sefi- Medical Instruments; Israel) and by the recommendation of a Sri Lankan Medical Council registered Medical Laboratory Technologist. Briefly, an aliquot of semen with a minimum 2.5 ml volume was placed in the chamber, and the numbers of motile and non-motile sperm were counted in three strips of 10 squares. In addition, the same process was repeated but on three different aliquots from the same ejaculate following the manufacturer's recommendation (Sefi- Medical Instruments; Israel). Also, the mean, fifth percentile and 95% CI were calculated for the numbers obtained in all three strips of 10 squares and from all nine strips of 10 squares.  $P < 0.05$  was considered statistically significant.

## Results

The comparison of the three different aliquots' mean obtained from the same for all three variables was demonstrated in Table 1.

**Table 1- Comparison of the three different aliquots mean from the same ejaculate for all three variables**

Sperm	Drop 1	Drop 2	Drop 3	P value
<b>Progressive Motility (%)</b>	37.1 ± 13.5	35.6 ± 11.9	34.7 ± 12.3	0.832
<b>Motility (%)</b>	41.6 ± 15.0	40.1 ± 13.3	38.6 ± 14.6	0.810
<b>Concentration X10<sup>6</sup>/ml</b>	60.7 ± 41.0	63.8 ± 47.9	59.7 ± 46.4	0.957

The mean and fifth percentile plus 95% confidence interval were calculated for the ejaculates analysed and compared with the fifth percentile minus 95% CI established as reference limits from more than 3300 fertile ejaculates by the World Health Organization<sup>1</sup>. The results are detailed in Table 2.

**Table 2- Sample ejaculates motility distribution (5th percentile + 95% CI) compared with WHO minimum threshold**

Sperm	Mean	Maximum*	Fertile Threshold Minimum**	P-value
<b>Progressive Motility (%)</b>	35.0 ± 13.7	33.7 ± 13.6	29.0	0.5
<b>Motility (%)</b>	56.9 ± 14.9	55.5 ± 14.8	40.0	0.5
<b>Concentration X10<sup>6</sup>/ml</b>	93.4 ± 49.5	91.5 ± 48.9	15.0	0.8

\*Fifth percentile plus 95% confidence interval

\*\*Fifth percentile minus 95% confidence interval

## Discussion

Semen analysis is essential for assessing male fertility. Semen analysis differs from most clinical procedures in that it is often carried out by a technician who has been trained on the job with little to no continuous guidance. WHO recommendations serve as the foundation for global procedural standardisation and reference values (2).

Even though semen is comprised of a highly heterogeneous sperm population, in our study, the comparison of the three different aliquots collected from the same ejaculate did not reveal any significant variations between them as the mean value does not represent the variance due to sampling error (Table 1). Sampling errors can occur even when sperm are properly combined if the ratios are off. Since only the cells visible in the microscopic field are counted when an ejaculate is investigated, whether manually or with the aid of a machine, the outcomes from a single sample of a semen ejaculate are diverse and unpredictable. As a result, sperm of various qualities can frequently be seen in different samples of the same ejaculate. As a result of this sampling error, it is impossible to characterise a man's semen quality and fertile potential.

It is recommended that at least two different ejaculates, at a minimum, should be analysed for diagnosis of male factor infertility (4,5,6). However, the American Society for Reproductive Medicine (ASRM) recommends a second semen analysis only if the first semen analysis is abnormal (7). In contrast, due to inherent variability between ejaculates, the World Health Organization and Chui et al. recommend two different ejaculates regardless of initial quality since they reported discordance between ejaculates, especially if the first analysis was normal than among men whose first sample had abnormal values based on the calculated predictive value (2,8).

In addition, it is advised that the sample be completely mixed before aliquots are obtained for assessment and that the outcomes of replicate aliquots must be consistent before the values are accepted. The Poisson distribution for sperm quantities and the binomial distribution for percentages are used to determine agreement between replicates (9).

Similarly, the mean and fifth percentile plus 95% confidence level were not significantly different (Table 2), suggesting that the mean value could be compared to the 2021 WHO reference values when interpreting the results.

## Conclusion

Seminal fluid analysis is a key investigation in male infertility workup. Interpretation of seminal fluid

analysis is pivotal in managing male factor infertility. Therefore, based on our study's findings, we conclude that when using the Makler counting chamber, the mean value calculated from one aliquot per ejaculate counted on three strips of ten squares should be acceptable. At least two ejaculates should be analysed when interpreting the results based on 2021 World Health Organization reference limits.

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