SEMEN ANALYSIS; CORNER STONE IN EVALUATING INFERTILITY

Aysha Khan, Zunera Sajjad, Shagufta Yousaf, Abeera Ahmed, Fatima Sana, Fawad Sana

Combined Military Hospital Kharian/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To study the patterns and distribution of various abnormal semen parameters in infertile males. *Study Design*: Cross sectional study.

Place and Duration of Study: Department of Pathology, Combined Military Hospital, Karachi, from Nov 2019 to Oct 2020.

Methodology: The study included 364 patients who presented with primary and secondary infertility. Consecutive convenient sampling was done. Semen analysis was performed using World Health Organization (WHO) latest guidelines. Samples were categorized as normospermia, azoospermia, oligospermia, asthenozoospermia and necrospermia.

Results: The study comprised of 364 samples of infertile males. Normal sperm count was observed in 317 (87%) males, azoospermia in 28 (7.6%) and oligospermia in 19 (5.2%) males. Low ejaculated volume and higher non-motile sperms were noted in oligospermia samples in comparison with normospermia samples. Asthenozoospermia was observed in 102 (28%) and oligoasthenospermia was noted in 15 (4.1%) samples.

Conclusion: Good quality semen analysis is a corner stone to diagnose the cause of male infertility.

Sperm concentration and motility are the important markers of normal male reproductive system and are related to each other.

Keywords: Asthenozoospermia, Azoospermia, Necrospermia, Oligospermia, Sperms.

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INTRODUCTION

Semen analysis is a basic test carried out in an infertile couple who are living together for at least one year with healthy sexual relationship, but are unable to reproduce a child without using any contraceptive measures.¹ Approximately 10-15% of couples suffer from infertility throughout the world. Male factor is responsible for 45% of such cases.² However, in our society females have to endure the infertility trauma and undergo wide range of tests, including invasive procedures causing physical and emotional trauma.

Semen analysis remains the basic and fundamental test in evaluation of infertility. Not only it provides an insight into fertility but also if used in broader sense, it can help in evaluation of male reproductive system and may give a clue about any serious underlying disorder. Semen analysis may also be requested for evaluation of hypogonadism, follow up after vasectomy, prior to donation for artificial insemination and storage of semen before radiotherapy etc.³

Semen analysis also reveals important information about sperm production, its motility and viability, patency of male genital tract, secretion of accessory

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organs as well as ejaculation and emission.

Semen consists of spermatozoa suspended in fluid like material necessary for its nourishment. Only sperms and 5% of the total volume of semen are produced in testes. About 40-80% of the secretions come from seminal vesicles, which contain fructose necessary for nourishment of the sperms. Out of 13-33% of the bulk comes from prostate that contains a number of enzymes including citrates, calcium, zinc etc. Approximately, 2-5% of the volume is also contributed by bulbourethral and urethral glands that contain mucoproteins and immunoglobulin A. The ability to penetrate the egg is also acquired in epididymis. White blood cells (WBCs) may also be present in semen. If in excess, they are referred to as leukocytospermia or pyospermia that may indicate infection of the genital tract. WBCs are also known to adversely affect motility of the sperms. Thus, the disease in any of these parts may have a significant effect on quantity and quality of the semen.4

The aim of this study was to assess different patterns and distribution of abnormal semen parameters in infertile males.

METHODOLOGY

This cross sectional study was conducted at the Department of Pathology, Combined Military Hospital

Correspondence: Dr Aysha Khan, Department of Haematology, Combined Military Hospital, Malir Pakistan

(CMH) Malir, from November 2019 to October 2020. The study included 364 male patients that presented to the male outpatient department (OPD).

Inclusion Criteria: Male patients between ages of 20-55 years, with the history of primary or secondary infertility were included in the study.

Exclusion Criteria: Individuals who did not give consent or did not come in the specified time i.e. within 3-7 days of coitus, were also excluded.

Sample was collected by non-probability consecutive sampling technique with the help of World Health Organization (WHO) sample size calculator. While keeping Confidence interval at 95 %, margin of error of 5% and prevalence at 15 %, sample size was calculated as 196.⁵ The study was conducted after approval by the Ethical Committee of CMH Malir [31/2020/trg]. The patients selected for the study were referred by the gynecologists for workup of primary as well as secondary infertility at the Department of Pathology, CMH Malir. All the subjects fulfilling the inclusion criteria were explained about the study. Informed consent was taken and patients' particulars were endorsed on a performa.

Semen sample was collected in wide mouth sterile bottle after 3-5 days of abstinence from coitus. Sample collection was preferred in laboratory; however, some of the samples were also produced at home and quickly delivered to laboratory in less than an hour time. A questionnaire was filled at time of receiving of the sample. Information was gathered regarding age, duration of marriage, first or second marriage, and whether the couple is living together. In case of secondary infertility, age of last childbirth, use of drugs/medicine and any other systemic illness/surgery taken place.

WHO 2010 parameters 3 were followed while examining these samples. Macroscopically, appearance liquefaction, pH and volume were noted. Normal colour was taken as grey or dirty white, liquefaction time 20 min, pH 7.2-8 and volume more than 1.5 ml were taken as normal. Microscopically, sperm concentration of >15.0 x 10⁶ spermatozoa/ ml, motility >40% (both progressive and non-progressive motility), >4% normal heads and vitality of >58% at 1st hour was taken as normal.

Following terms were used using WHO criteria 3; Azoospermia: Absence of sperms in ejaculate, oligospermia: sperm count below 15x10⁶, asthenozoospermia: reduced sperm motility and necrospermia: dead sperms. Sample was placed at 37°C and was analyzed within one hour. Observation was made for abovementioned parameters both macro and microscopically. All the relevant data was analyzed through Statistical Package for the social sciences (SPSS) version 23. Numerical variables were presented by mean ± SD. Categorical variables were presented by frequency and percentage.

RESULTS

A total of 364 samples were included in this study. Mean age of patients was 30.8 years ± 5.0 years. Primary infertility was present in 295 (81%) while secondary infertility was present in 69 (19%) patients. Out of 364 samples 356 (97.8%) had normal semen volume, 06 (1.6%) showed hypospermia while 2(0.5%) showed hyperspermia (Table-I). Sperm concentration was normal in 317 (87%) samples and oligospermia in 19 (5.2%) samples (Table-II). No abnormality was found in 210 (57.7%) samples. Most common abnormality was asthenozoospermia observed in 102 (28%) of patients, followed by azoospermia 28 (7.7%), necrospermia 5 (1.4%), oligoasthenospermia 15 (4.1%) and oligospermia 4 (1.1%) samples (Table-III). No abnormality of liquefaction was found in any sample. Pus cells were present in 75 samples.

Table-I: Distribution of sperm volume.

Sperm volume	Frequency (%)
Normal volume (1.5-4.5 ml)	356 (97.8)
Hypospermia (< 1.5 ml)	6 (1.6)
Hyperspermia (> 4.6 ml)	2 (0.5)

Table-II: Frequency of sperm concentration.

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Sperm Concentration	Frequency (%)
Normospermia	317 (87.2)
Oligospermia	19 (5.2)
Azoospermia	28 (7.6)
Table-III: Distribution of semen analysis parameters.	
Semen Analysis Parameters	Frequency (%)
Essentially Within normal limits	210 (57.7)
Asthenospermia	102 (28.2)
Azospermia	28 (7.6)
Necrospermia	5 (1.4)
Oligoasthenospermia	15 (4.1)
Oligospermia	4 (1.1)

DISCUSSION

Infertility is a taboo that targets only the female population in our society and has a considerable impact on their mental and psychological health although males equally contribute towards this problem. Advanced knowledge of reproductive techniques provides wide range of treatment options, giving light of hope for infertile couples. Semen analysis is the fastest, easiest, cost-effective and reliable test giving a large amount of information without any invasive test.

Primary and secondary infertility both are common in our part of the world, contrary to belief that if men have fathered a child once, they remain fertile throughout their lives. In the study by Aulia *et al*⁶ and Haifa and Turki,⁷ primary infertility was reported to be 88.7% and 80.5% while secondary infertility was 11.3% and 19.5% respectively.

The mean sperm count in our study was 83.5 ± 6.5 million which was comparable to the study in UK, in which semen analysis was performed on a population of 1801 suspected infertile men, in which the mean sperm density was 84.3 ± 7.89 , contradicting the fact that sperm count is declining over the years as predicted by Carlsen *et al*⁹, mean sperm count as reviewed by Virtanen *et al* is 41-55 x 10⁶/ml showing a downward trend.¹⁰

Considerable attention was paid to the study of Carlsen et al that over the past 50-60 years, globally sperm density has decreased by about 50%.9 Similar study was carried out in Wuhan city of China that concluded declining sperm count in their country.¹¹ In another study conducted in India by Mishra et al to confirm the hypothesis of declining sperm count in men also shows decline in sperm concentration.12 However, Elia *et al* performed a comparative study of seminal parameters between samples collected in 1992 and 2010 stating no change in sperm count over these years.¹³ Dietary patterns might lead to decline in sperm count as concluded by study of Anna et al in Poland.14 In Pakistan situation is not as devastating as in other parts of world due to low frequency of urogenital infections, testicular cancers and limited environmental exposure/eatables containing estrogen like activity hindering spermatogenesis. Our study gave some insight over the situation in Pakistan. Although study was conducted in one year and longer duration studies are required to confirm the situation in Pakistan.

Our study revealed azoospermia in 28 (7.6%) of males, which was comparable to study by Jairajpuri *et al* from Delhi in which azoospermia was 9%¹⁵ and Ramya *et al* reported 7.5%.16 Much lower percentage was reported by Anuja *et al* from Devanahalli as 2%.¹⁷ One of the Pakistani study by Fouzia *et al* shows Azoospermia to be 14.89%.⁵ Much higher percentage of azoospermia is also recorded in Jakarta as 24.4%.⁶ It may be due to referral center where only confirmed infertility cases visit.

Oligospermia (sperm count less than 15x10⁶ml) was reported to be 5.2% in our study. Abnormalities in accessory sex glands fluid synthesis may lead to low ejaculated volume. Seminal vesicle produces about 70% of seminal plasma. Physical obstruction and retrograde ejaculation may also contribute to low ejaculated volume. Anuja *et al*¹⁷ reported 6% oligospermia which was comparable to our study, while Fouzia *et al* reported 5 (11.11%) same as 13.3% by Chituri *et al*.¹⁶ A very high percentage of oligospermia as 39.7% is reported by Aulia et al from Jakarta may be for the same reason as they reported higher percentage of azoospermia.⁶

Mean ejaculated volume in normopspermia was 3.25 ± 1.35 vs 2.05 ± 2 in oligospermia and 2.43 ± 1.19 in azoospermia sample respectively. Low ejaculated volume is defined as <1.5% ml. Majority of our patients had normal semen volume 97.84%, while 1.6% showed hypospermia (<1.5ml), and hyperspermia in 0.5%. These results were comparable to a study conducted in Delhi,¹⁰ and Dewanhalli,¹⁷ which revealed 91% and 92% normal semen volume, while 4.9% and 7% having hypospermia (less than 1.5ml) respectively. Similar results are also shown by Fauzia et al.⁵ The adequacy of semen volume in our study could be the result of sexual abstinence of 3-4 days. Our study proposes that 3-4 days of sexual abstinence is required for proper collection and accurate analysis of semen. The volume plays little or probably no role in etiology of male infertility.

Another morbidity factor is male genital tract infections. It is known to affect not only the quality but also the environment that provides nourishment to sperms.

Epididymal proteins and other substances produce some conformational changes in sperms. They greatly influence the motility of sperms, which enhances while passing through epididymis. Therefore, sperm motility is an essential factor required for sperm to travel a long distance to fertilize the oocyte. Sperm motility is the pivotal factor of epididymal functioning. Asthenozoospermia is the most common abnormality found in our study. The mean percentage of normal motile sperms was 57% ± 9.76 in normospermia samples as compared to oligospermia in which motile sperms were 28% ± 10%. In our study, asthenozoospermia was observed in 28% of samples and comparable results were obtained from a studies conducted by Butt et al⁵ (25.81%) and Aulia et al⁶ (22.1%), while much lower percentage is reported by Jarajpuri et al¹⁵ (5.9%), Ramya et al^{16} (4.4%) and by Anuja et al^{17} (3%).

Another morbidity factor is male genital tract infections. It is known to affect not only the quality but also the environment which provides nourishment to sperms.^{18,19} In our study, we couldn't link any bacterial infection having direct relationship with sperm count or sperm motility. However, we are still working in collaboration with microbiologist with large sample size and results will be available soon.

CONCLUSION

Good quality semen analysis is a corner stone to diagnose the cause of male infertility. Concentration and motility of sperms are the main parameters that indicate normal male reproductive functioning.

Conflict of Interest: None.

Authors' Contribution:

AK: Concept, design, ZS: Data analysis, SY: Data interpretation, AA: Intellectual, FS: Data analysis, FS: Data analysis.

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