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Original Research Article

Diagnostic value of semen analysis in male infertility

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ABSTRACT

Background: Semen analysis is an indispensable diagnostic tool in the evaluation of the male partners of infertile couples. Aim was to analyze semen parameters according to WHO criteria (5th edition) in patients attending infertility OPD and compare it with 6th edition.
Methods: It was a retrospective descriptive cross sectional study that took place at the department of pathology, JMF ACPM medical college, Dhule, Maharashtra. This study included 103 subjects who were presented for semen analysis from June 2022 to June 2024. The data regarding ejaculate volume, count, motility and morphology were collected.
Results: Abnormal semen quality was a major factor of infertility in our rural setup with 39% of male partners of infertile couples having abnormal semen parameters.
Conclusions: Male contribution towards infertility is yet to be studied and requires more elaborate research.

Keywords: Azoospermia, Infertility, Semen

INTRODUCTION

Infertility can be defined as a failure in achieving a successful pregnancy of a couple after one year of regular sexual intercourse without using protection or contraceptive methods. It is a global health problem in the community with physical, psychological and social influences.[1] Primary infertility is the case, when the man has never impregnated a woman. In India, the prevalence of primary infertility has been estimated to be 10-20%. Secondary infertility implies when the man has some time impregnated a woman, even if the women are not the partner in the present couple.[2]

Infertility has been an on-going concern through the ages and remains a major clinical problem today, affecting 15-20% of couples. Male infertility accounts for 40-50% of infertility, affecting 7% of all men.[3]

Infertility in a male is assessed by taking a detailed medical and sexual history, a complete physical examination, and semen analysis. In 1677, firm evidence of the contribution of the sperm to reproduction came when Leeuwenhoek, on examining his own ejaculate, saw under the microscope live human sperm cells in a drop of semen for the first time.[4]

Semen analysis is an indispensable diagnostic tool in the evaluation of the male partners of infertile couples. It is a procedure in which specialists examine and evaluate the health, vitality and overall quality of a man's semen and sperm.[5]

Careful evaluation of the ejaculate parameters may suggest the possible causes of infertility and their identification could help to institute appropriate therapy, if available.

World Health Organization (WHO) manuals have served as a primary reference for seminal fluid analysis procedures since long. WHO has published five editions of the manual for semen analysis at different times and the 5th edition was published in 2010. The standards and reference value for different parameters have been defined again with each subsequent new edition. According to us, initial 4 editions were based on consensus by experts, but fifth edition is evidence based and hence superior to initial 4 editions.[6]

However, in 2021, the World Health Organization (WHO) released its 6th edition of semen analysis manual.

This 6th manual facilitates laboratory excellence by providing detailed instructions to improve the overall calculations and interpretations of the semen parameters. Also. the 6th edition emphasizes that the use of the 5th centile values of basic semen parameters alone is not sufficient to diagnose male infertility and that further clinical and/or laboratory evaluation of the patient is needed based on the judgement of the treating physician.

Semen parameters vary from time to time in the same individual as any other fluid parameters. Semen sample is collected by masturbation with an abstinence period of two to seven days, usually near the laboratory premises to reduce the time interval between collection and analysis of sample as the parameters quickly change over time. The gross findings of the semen sample, such as the volume of semen, pH of semen, color of semen, liquefaction time and viscosity are measured carefully. The sample is then further evaluated under a light microscope to determine the motility, vitality, concentration, and morphology of sperms. According to WHO manual 2010, semen volume more than 1.5 ml, sperm concentration more than 15 million, progressive motility more than 32%, more than 58% live forms with more than 4% normal forms defines reference range for semen analysis. Many studies have proved that the total motile sperm count (volume x concentration x motility) is the most predictive factor in assessing fertility of male as compared to volume of semen, concentration of sperms, and motility of sperms counted individually.

Table 1: Comparison of semen analysis parameters: WHO 2010 versus WHO 2021.

Parameters	WHO 2010	WHO 2021
Semen volume (ml)	1.5 (1.4-1.7)	1.4(1.3-1.5)
Total sperm number (106 per ejaculate)	39 (33-46)	39 (35-40)
Total motility (%)	40 (38-42)	42 (40-43)
Progressive motility (%)	32 (31-34)	30 (29-31)
Non progressive motility (%)	1	1
Immotile sperm (%)	22	20 (19-20)
Vitality (%)	58 (55-63)	54 (50-56)
Normal forms (%)	4 (3-4)	4 (3.9-4)

However, the latest 6th edition of WHO manual entitles robust evaluation and processing of human semen. The summary of changes in the 6th edition of WHO manual of human semen analysis compared to the previous 5th edition is tabulated in Table 1.

The aim of this study was to investigate the semen quality in men seeking infertility evaluation, in terms of the sperm concentration, total sperm motility, sperm morphology and incidence of azoospermia at a regional level over a period of 2 years.

Aim and objectives

To analyze semen parameters according to WHO criteria (5th edition) in patients attending infertility OPD and compare it with 6th edition.

METHODS

Type of study

It was a retrospective descriptive cross-sectional study.

Duration of study was 2 years from June 2022 to June 2024.

Place of study

The study took place at the department of pathology, JMF ACPM medical college, Dhule, Maharashtra.

Study population

Male partner of the infertile couples coming to laboratory for semen analysis were the study population.

Sample size

Total 103 male participants were included in the study.

Inclusion criteria

Infertile couples who were living together for more than one year and had regular unprotected sexual intercourse. Only those male patients were considered for the study

whose partners were not having any abnormality in fertility evaluation.

Exclusion criteria

Previous disease or surgery associated with reproductive function (including varicocele, cryptorchidism, epididymitis, mumps, and azoospermia); vasectomy and vasectomy reversal. Couples with female factor infertility. Couples not living together. Men who refused to do semen analysis.

Methodology

Total 103 couples reaching infertility OPD were considered for this study. According to the inclusion and exclusion criteria, men were called for semen analysis after 3 days of abstinence. The semen samples were collected in laboratory in clean sterile containers by masturbation. These samples were then processed for gross and microscopic examination. Detailed history like addiction, occupation, history of previous issue was noted. All patients with past history of smoking/tobacco chewing or present smokers/tobacco chewers were considered as smokers/ tobacco chewers. A systematic report was made for each patient according to WHO manual for semen analysis fifth edition and then it was compared with the 6th edition. Gross examination was noted as volume, colour, pH, liquefaction time and viscosity. For microscopic examination, wet mount study, Neubauer's chamber for counting and eosin-nigrosine smears for vitality was done. Results obtained were tabulated and analysed. 41 (39.80%) patients had abnormal seminogram. The abnormal samples are further analysed.

RESULTS

The present study was conducted to determine the abnormalities in semen samples of male infertility. Of the total 103 semen samples studied, 41 (39.80%) had abnormal seminogram. The abnormal samples were further analysed. Majority of them were in the age group of 31-40 years (60.97%) followed by 21-30 years (29.26%) and then in 41-50 years (7.31%) while a single case was seen in more than 50 years age group.

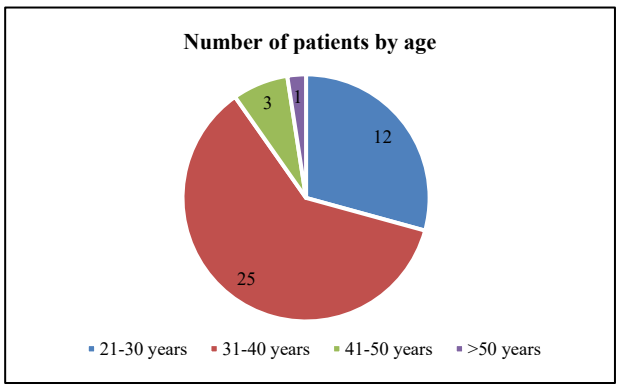

Figure 1: Number of patients by age.

Majority i.e. 63.41% patients had duration of infertility below 5 years, 34.14% between 5-10 years and there was a single case (2.43%) with more than 10 years of infertility.

Table 2: Duration of infertility.

Duration of infertility	Number of cases (%)
<5 years	26 (63.41)
5-10 years	14 (34.14)
>10 years	01 (2.43)

58.53% were having some form of substance abuse; of which 33.33% were smokers,29.16% were addicted to tobacco and 37.5% were addicted to alcohol.17 males (41.47%) had no substance abuse.

Table 3: Distribution of cases according to substance abuse.

Addicted to	Number of cases (%)
Smoking	8 (33.33)
Tobacco	7 (29.16)
Alcohol	9 (37.5)
No abuse	17 (41.47)

Out of the 41 samples analysed in the current study, 4 individuals (9.75%) had an ejaculate volume less than 1.5 ml, while 37(90.24%) had ejaculate volume of more than 1.5 ml. However, according to the 6th edition of the WHO 2021 the lower 5th percentile of the semen volume has been reduced to 1.4 ml from the previous 1.5 ml. In this study 3 individuals had ejaculate volume of less than 1.4 ml while 38 individuals had ejaculate volume of more than 1.4 ml.

Colour, pH and liquefaction time of all the samples analysed was normal.

13 patients (31.70%) had sperm count <20 million,5 patients (12.19%) had sperm count between 20-60 million, while 23 patients (56.09%) had sperm count >60 million. The minimum total sperm number (106 per ejaculate) remains the same in the new WHO manual (6th edition) of semen analysis.

In our study, 12 patients (29.26%) had <25% motile sperms/hpf, 8 patients (19.51%) had 25-50% motile sperms/hpf, 18 patients (43.90%) had a motility rate between 50-75%/hpf while 3 patients (7.31%) had 75-100% motility rate /hpf. The new WHO 2021 has revised the total motility of the sperms (in %) from 40 to 42. In our study 26 patients (63.41%) had >32% progressive motility while the remaining had <32% progressive motility. Additionally, in the 6th edition, the classification of sperm motility is reverted to the previous 5th edition by distinguishing progressive motile sperm into rapid and slow. The rationale for this change is that the presence of rapidly progressive motile sperm can affect the outcome of fertility. However, the limitation of this study was we could not classify the motility according to the new

criteria. The 6th edition suggests total sperm motility below 40% as an indication for sperm vitality assessment.

16 patients (39.02%) had normal morphology and 25 patients (60.97%) had abnormal morphology. Any defects of head, neck, mid piece and tail were considered as abnormal morphology.

There are no changes in sperm morphology assessment or interpretation in the 6th edition.

Pus cells were present in nearly half of the patients with abnormal seminogram (n=19, 46.34%).

Table 4: Spectrum of seminal parameters of the study participants.

Seminal parameters	Number of patients	Percentage
Volume (ml)		
<1.5	04	9.75
>1.5	37	90.24
Count (million/ml)		
<20	13	31.70
20-60	05	12.19
>60	23	56.09
Proportion of motile sperms in percentage		
<25	12	29.26
25-50	08	19.51
50-75	18	43.90
75-100	03	7.31
Abnormal morphology		
Present	25	60.97
Absent	16	39.02

DISCUSSION

Childbearing is considered as an essential part of living and yardstick by which women's worth is measured especially in a developing country. So, infertility invites social stigma. However, recent researches have proved that problem is not gender specific. Infertility can be attributed to male factor, female factor or a combination of both. The awareness of magnitude and importance of male factor infertility is relatively recent. Semen analysis remains the cornerstone to investigate male infertility.

"Male factor" infertility (MFI) is considered as a change in sperm concentration and/or motility and/or morphology in at least one sample of two sperm analyses, collected 1 and 4 weeks apart. WHO (2010) manual for semen analysis have changed nomenclature from "normal" to "reference values" with respect to sperm concentration, motility, morphology and all other semen parameters. Males with sperm parameters below the WHO normal values or reference values are considered to have male factor infertility.[7]

The present study was conducted to determine the abnormalities in semen samples for detection of male infertility. There are very few studies in rural areas as far as infertility is concerned. So many researches and my study also prove that not only female but males are also responsible for infertility. Thus, screening of males by simple semen analysis test gives an idea about the pathological infertility problems.

Abnormal seminogram was present in 39.80% patients in this study. Priyakshi et al got 37% abnormal seminogram, Ramya et al and Jajoo et al found 52% abnormal seminogram while it was 62.7% in the study done by Hemlata et al.[5,8-10]

In the present study, majority (60%) of the patients were in the age group of 31-40 years. This was similar to the studies done by Surekha et al and Keyuri et al who also found the maximum number of patients in the same age group.[7,11] The next most common age group in our study was 21-30 years followed by 41-50 years and the least common being more than 50 years. This was in concordance with the study done by Hemlata et al 5 who also found similar pattern of the age group distribution. The effect of women's age on fertility is well recognized, whereas in men's age, it remains uncertain. It concludes that age contributed to a decline in sperm motility and morphology in men over age of 40 years.

Majority of the patients had duration of infertility less than 5 years (63.41%), 34.14% had duration of infertility between 5-10 years while 2.43% patients had more than 10 years of infertility in our study. This agrees with the studies done by Atul et al, Jajoo et al and Hemlata et al.[4,5,10] The duration of infertility less than 5 years in their studies were 66%, 62% and 73.9% respectively. The duration of infertility between 5-10 years were 20%, 32% and 24.32% respectively. The duration of infertility more than 10 years were 14%, 6% and 2.7% respectively.

In our study 58.53% men had history of substance abuse; of which 33.33% were smokers, 29.16% were tobacco chewers and 37.5% were alcoholics. Priyakshi et al found 42% men as substance abusers.[8] Anuja et al in her study found 47% smokers, 13% alcoholics and just 1% tobacco chewers.[3] According to a study by Samal et al, the abnormality of the semen analysis report was found in 35.49%, 86.49% and 53.75% in smokers, alcoholics and in combinations of these addictions respectively.[12] This shows that cigarette smoking has detrimental effects on spermatogenesis and they thereby impair fertility. Hence men should be encouraged to stop smoking especially while trying to conceive. Excessive alcohol consumption has been associated with poor reproductive function. Alcohol has profound effects on Leydig cell function by reducing testosterone synthesis and its metabolite, acetaldehyde, causing membrane damage and the formation of Leydig cell auto antibodies.

Colour, pH and liquefaction time of all the samples analysed was normal in our study.

Colour of semen has some significance in semen examination. Normally liquefied semen shows homogeneous, grey-opaque appearance and appears less opaque if the sperm concentration is low. The red colour is seen in hemospermia and yellow colour in a man with jaundice or taking certain vitamins/drugs or more abstinence period.

When collected, semen appears as mass of thick coagulum. After few minutes, prostatic proteases liquefy semen and it appears watery. This usually happens within 15 minutes or sometimes takes up to 60 minutes. The initial part of semen is rich in sperms and prostatic fluid while later part is rich in seminal vesicular fluid. Hence, the initial part of semen is very important to correctly calculate liquefaction time. Delayed liquefaction (>60 minutes) is noted and indicates pathology. These samples usually show high viscosity.

As we think about semen volume, majority (90.24%) men had volume of more than 1.5 ml while 9.75% men had less than 1.5 ml semen volume. This correlated with the studies done by Nandini et al and Surekha et al who got 7.45% and 16.7% men having less than 1.5 ml semen volume in their study respectively.[7,13] However, Keyuri et al got 44.75% men having less than 1.5 ml semen volume.[11] Lower values are seen in obstruction of the ejaculatory duct, congenital bilateral absence of the vas deferens (CBAVD), retrograde ejaculation or collection problems. The problems associated with collection can be reduced by counselling patient before the collection of samples. In case of retrograde ejaculation, a post ejaculate urine examination is necessary for detection of sperms in urine.

Sperm count <20 million/ml was found in 31.70% patients in our study, count between 20-60 million/ml was found in 12.19% while count >60 million/ml was found in 56.09% patients. Atul et al found 70% patients with count <20 million/ml.[4] Jajoo et al found 25% people with count <20 million/ml, 34% with count between 20-60 million/ml while 41% people had count >60 million/ml.[10]

Decreased motility (less than 50%) was seen in 48.77% patients in our study. Ramya et al found 24.5% patients with decreased motility, Jajoo et al found 35% patients with decreased motility while it was 94% in the study by Atul et al.[4,9,10] Less than 25% motility was seen in 29.26% patients in our study. This was 37.83% in the study by Hemlata et al and 46% in the study by Atul et al.[4,5]

The progressive motility is important indicator of male fertility and is related to pregnancy rates. A Progressive motility is defined as spermatozoa moving actively, either linearly or in a large circle, regardless of speed. All other forms are considered as non-progressive motility while non-moving sperms are classified as immotile. >32% progressive motility was seen in 26 patients (63.41%) in our study. This was 54.1% in the study by Rahul et al and 80.7% in the study by Surekha et al.[6,7] Non progressive motility was seen in 36.59%in this study which was 19.3% in the study by Surekha et al.[7] Motility is one of the most important factors of male infertility.

Morphology of sperms is very important clue for male fertility. Many morphological abnormalities are seen in sperms like abnormality of head, middle piece and tail. Some form of abnormality is seen in almost all semen samples. But it is the count of abnormal cells that matters. An arbitrary limit of 4% is set by WHO for abnormal sperm cells. Abnormal morphology was present in 60.97% samples in our study while 39.02% samples had normal morphology. Our study agreed with the studies done by Atul et al , Jajoo et al and Hemlata et al who found 72%, 65.9% and 78.37% sperms with abnormal morphology in their studies respectively.[4,5,10]

Pus cells were present in 46.34% samples in our study. Nandini et al found 12.42% samples with pus cells and Rahul et al found 17.6% samples with pus cells.[6,13] This was a little higher in the study by Hemlata et al who found 61% samples with pus cells in their study.[5]

A high-quality basic semen analysis is the cornerstone of investigations related to infertile couple, but it is important to acknowledge the limitation of semen analysis with respect to collection, processing, evaluation, biological variation of the parameters and lack of information on sperm function. The conventional semen parameters, such as sperm concentration, motility and morphology, are markers of male reproductive function. However, due to limitations as above, a normal semen analysis does not guarantee the fertilization potential of sperm. Though it not a test of fertility, but it does provide information about abnormalities of sperm count and morphology. Optimal age of marriage, refraining from addictions, timely medical assistance can help the couples to have successful pregnancy.

CONCLUSION

Semen analysis is a very important tool for evaluation of male infertility and in expert hand; it will provide vital information to clinician. Abnormal morphology of sperms should be noted precisely. History of addiction provides important clue to pathologist during semen analysis and should not be neglected. Males contribute towards infertility in couples significantly and further study and assessment is required to accurately predict the importance of this. This study has demonstrated that abnormal semen quality is a major factor in our rural setup with 39% of male partners of infertile couples having abnormal semen parameters.

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