IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 23, Issue 3 Ser. 5 (March. 2024), PP 25-29
www.iosrjournals.org

Study Of Semen Analysis In Male Partners Of Infertile Couples At A Tertiary Care Center

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Abstract:
Background*:Semen analysis has remained an objective, inexpensive, readily available an indispensable diagnostic tool in the evaluation of the male partners of infertile couples. This test has been standardized throughout the world through the World Health organization (WHO) by producing, editing, updating and disseminating a semen analysis manual and guidelines.*
Aim and Objectives : *This study assesses the pattern of semen analysis results in male partners of infertile couple.*
Materials and Methods*: A retrospective cross sectional study on 106 semen samples of male partners of infertile couple were analyzed by manual methods and carried out between 1st January to 31st December 2023.*
Results*:This study, done at Dr.ShankarraoChavan Government medical college, tertiary care centre has demonstrated that abnormal semen quality is a major factor contributing to infertility in couples, focusing on volume, sperm count, motility, morphology and presence of pus cells. Among the participants, the majority (70.8%) had a semen volume ranging from 2-4 ml, while 70% exhibited a sperm count of less than 20 million.Motility analysis revealed that 94% had motility below 50%, with 71% showing abnormal sperm morphology.Additionally 32.1% of subjects presented with pus cells.*
Conclusion:*Semen analysis is the keystone of infertile couple. Semen parameters such as sperm concentration, motility and morphology are indicator for male reproductive function. In this study both sperm quantity and quality were more affected when compared to similar studies. Only 36 % of analyzed sample had normal semen parameters..*
Key Word*:Male infertility, Semen analysis, Azoospermia.*

Date of Submission: 13-03-2024 Date of Acceptance: 23-03-2024

I. Introduction

A firm evidence of the contribution of the sperm to reproduction came when Leeuwenhoek in 1677, while examining his own ejaculate, under the microscope saw living human sperm cells in a drop of semen for the first time. Infertility is a unique medical condition because it involves a couple, rather than a single individual. It has significant psychological, sociocultural, economical, demographic, and physical problems.[1,2]

Infertility is clinically defined as an inability to be pregnant after 12 months or more of regular unprotected sexual intercourse.[3–5] Infertility can be primary or secondary. Primary infertility describes women who have not been conceived previously. In secondary infertility, there is at least one conception but fails to repeat.[1,3–5] It is widely accepted that male factor alone accounts for infertility in about 40% cases, female factor alone in 40% of the cases of infertility, and in 20% cases, there is combined male and female factor.

According to the records from the World Health Organization (WHO), about 40% of infertility cases are due to male factors which are due to aging processes that lead to decrease sperm motility, sedentary work, and lack of exercise.[6] Other factors are infection and oxidative stress and an increase in inflammatory cytokines in seminal plasma that decreases sperm quality and damage sperm DNA.[7,8] Nutritional factor had an important role in sexual health and semen quality, especially Vitamin D deficiency.[9] Semen or sperm analysis after 3 days of abstinence is usually the first laboratory test that is done and one of the most important test for fertility tracking and follow up. The characteristics of semen analysis are an abnormality in sperm motility, PH, color, morphology, viscosity, semen volume, sperm concentration, and sperm count that done using visual examination, microscope, and counting chambers.[10]

This study aims to assess the semen quality and quantity, especially to evaluate the seminal pattern of the male partners of infertile couples.

II. Material And Methods

Study period and area: The study was conducted from 1st of January 2023 to 31st of December 2023, at Dr. ShankarraoChavan Government Medical College, Vishnupuri, Nanded, Maharashtra.

Study design: A facility-based retrospective cross-sectional study was conducted.

Study population-All male clients who attended GyanecologyOPD for workup of infertility and undergone semen analysis. History was taken from them regarding age, duration of marriage, first or second marriage, occupation, type of infertility, whether primary or secondary, drug intake, symptoms of any venereal infection, surgical, and medical history.

Semen Analysis: Patients were instructed to give a sample after abstinence from coitus for 3–4 days and collected aseptically by masturbation into sterile wide-mouthed containers within hospital. Semen analysis was performed according to the methods and standards outlined by the WHO.[11] All samples were incubated at 37 °C and analyzed within 30 min to 1 h of collection, then after liquefaction, the semen specimen was thoroughly mixed with the help of a pipette for the following parameters: volume, appearance, liquefaction, concentration, motility, morphology and viability and the presence of pus cells was assessed by microscope. Volume was measured with a graduated disposable pipette. The power of hydrogen (pH) value was measured using pH paper and compared with a calibration strip.

Sperm count was done in the hemocytometer after appropriate dilution. Motility was observed under microscope in wet preparation. Vitality test using the Eosin-Nigrosin stain was done for membrane intact spermatozoa. Number of stained (dead) and unstained (alive) spermatozoa was counted and results were percentage.

The parameter studied: Appearance (grey to opalescent); volume (2.0 ml or more); PH (7.2–7.8); sperm concentration (>15 × 10^6spermatozoa/ml); total sperm count (39 × 10^6or more/ejaculate); motility (50% or more with forward progression); morphology (4% or more with normal form); and white cell count or pus cell (<1 × 10^6/ml).

Terminologies: Semen samples were divided on the basis of sperm count per milliliter of semen in accordance with the WHO, 2021: Normospermia , oligospermia , and azoospermia.
The samples grouped were compared for ejaculated volume, pus cells, motility, and morphology. The following definitions were used according to the WHO,2021 definitions**(Table 1)**:Normospermia: Sperm count 15 million/ml to 120 million/ml., oligospermia: Sperm count below 15 million/ml., azoospermia: Absence of spermatozoa in the ejaculation,aspermia- no semen, asthenospermia: Reduced sperm motility, teratozoospermia: Abnormal sperm morphology, necrozoospermia- all sperm cells are non-viable,oligoasthenoteratospermia: All sperm variables abnormal

Table 1:WHO 2010 (5th Edition) and WHO 2021 (6th Edition) lower fifth percentile (with 95% confidence interval) of semen parameters from men in couples starting a pregnancy within one year of unprotected sexual intercourse leading to a natural conception

	WHO 2010	WHO 2021
Semen volume (mL)	1.5 (1.4–1.7)	1.4 (1.3–1.5)
Total sperm number (10^6 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 (1–1)
Immotile sperm (%)	22	20 (19–20)
Vitality (%)	58 (55–63)	54 (50–56)
Normal forms (%)	4 (3–4)	4 (3.9–4)

III. Result

Result of 106 semen sample which were analyzed at Dr.S.C.G.M.C. Nanded from1st of January 2023 to 1st of December 2023 based on WHO Guideline 2021 for semen analysis, out of which 64% were abnormal. These abnormal sample were further analyzed.

The age of study participant range from 20 to 40 year with mean age of the men in this study was 30 ± 8 year. The majority were between age group 25-30 year accounting for 40 % **(Figure 1).**

Majority i.e. 72% had duration of infertility below 3 years, 27% between 3-5 years and there was 7% with more than 5 years of infertility. **(Table 2)**

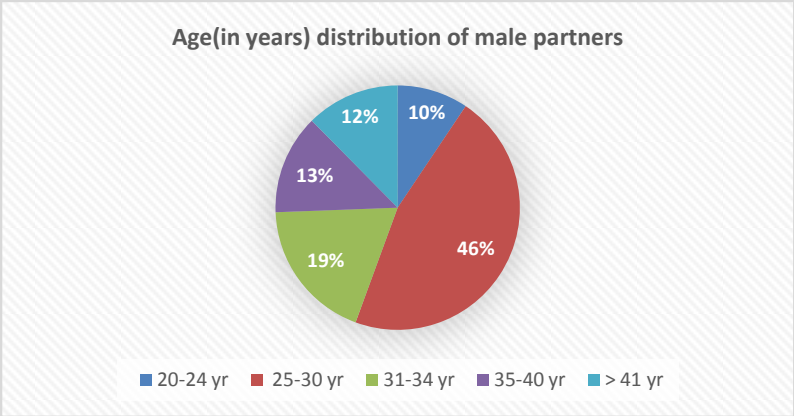

Figure 1. Age (in year) distribution of study participants at Dr. S.C.G.M.C. NANDED

Table 2.Baseline characteristics of study subject:

Characteristics	Number	Percent
Duration of infertility		
<3 Year	**72**	**68 %**
3-5 Year	**27**	**25 %**
>5 year	**7**	**7 %**

Table 3: % age proportion of abnormal sample:

% age proportion of abnormal sample	Number	Percent
20-24 Year	**04**	**5.9 %**
25-30 Year	**24**	**35.3 %**
31-34 Year	**19**	**27.9 %**
35-40 Year	**16**	**23.5 %**
≥41 Year	**05**	**7.4 %**

Among the men who had abnormal semen analysis, 5.9 % were between 20-24 year, 35.3 % were between 25-30 year, 27.9% were between 31-34 year, 23.5% were between 35-40 year and 7.4 % were above 41 year.**(Table 3)**

As far as semen volume is concerned, 27.3% male had volume < 2 ml, 70.8% had volume between 2-4 ml and only 1.9 % had volume > 4 ml.70% had sperm count < 20 million. In our study, 94% patient had < 50% motile sperm/hpf out of which 47% had < 25 % motile sperm,29% had normal morphology and 71% had abnormal morphology and pus cell present in 32% .**(Table 4)**

Table 4: Semen pattern among the study subject.

	Number	Percent
• **Volume**		
≤2 ml	**29**	**27.3 %**
2-4 ml	**75**	**70.8 %**
≥4 ml	**2**	**1.9 %**
• **Sperm count**		
≤20 million	**74**	**70 %**
≥20 million	**32**	**30 %**
• **Motility**		
≤50 % (≤ 25%)	**99 (47)**	**94% (47%)**
≥50 %	**07**	**06%**
• **Morphology**		

Normal	**31**	**29%**
Abnormal	**75**	**71%**
• **Pus cell**		
Present	**34**	**32.1%**
Absent	**72**	**67.9%**

According to 2021, WHO normal reference values for semen analysis, this study identified severe forms of semen analysis parameters. Only, 38 (36%) of analyzed samples were normozoospermic in which case all semen parameters were normal. The majority, 68 (64%) had one or more abnormal semen analysis parameters. Azoospermia 11 (16.2%), Oligozoospermia 39 (57.3%) out of which severe oligozoospermia 4 out off 39 (10.2%), teratospermia 5 (7.4%), necrozoospermia (4.4 %) and asthenozoospermia (14.7 %) were the severe forms of abnormal semen analysis findings detected in this study. **(Table 5).**

Table 5:Semen analysis finding in abnormal samples:

	Number	Percent
• **Azoospermia**	**11**	**16.2%**
• **Oligospermia (Severe oligospermia)**	**39 (4)**	**57.3% (10.2%)**
• **Teratospermia**	5	7.4%
• **Necrospermia**	**3**	**4.4%**
• **Asthenozoospermia**	**10**	**14.7%**

IV. Discussion

The result of this study provide valuable insights into the patterns of semen analysis results among male partners of infertile couples. The findings highlight several important aspects related to semen quality and quantity, shedding light on the prevalence of abnormalities in this population.

Table 6: Semen analysis parameters of this study compared to other studies.

Parameter	Current study 2024 (n=106)	Temesgen Tilahun et al. (n=131)	Onyebuchi Et al.[20] (n=376)	Aulia et al.[14] (n=1186)	Jairajpuri Et al.[17] (n=139)	Kurdukar Et al.[19] (n=40)
Normozoospermia	36%	16%	50.3%	33%	16%	55%
Oligozoospermia	57.3%	48.9 %	38.6%	39.5%	17%	30%
Azoospermia	16.2%	24.4%	11.7%	24.4%	9%	10%
Asthenozoospermia	14.7%	43.5%	23.4%	5.9%	22.1%	27.5%
Teratozoospermia	7.4%	27.5%	36%	2.6%	33.5%	0%
Necrospermia	4.4%					

Prevalence of abnormal semen parameters: The study found that a significant proportion of the analyzed semen samples (64 %) exhibited one or more abnormal semen analysis parameters.This indicates a high prevalence of semen abnormalities among male partners of infertile couples.Such abnormalities included azoospermia, oligozoospermia, teratospermia, necrospermia and asthenospermia with oligozoospermia being the most common abnormality detected.

Comparison with existing studies: Interstingly the prevalence of abnormal semen parameters in this study appears to be higher than that reported in similar studies. This suggests potential variation in semen quality and quantity among different population or may indicate the need for further investigation into environmental, genetic or lifestyle factors influencing male reproductive health in the study area.

Association with age and duration of infertility: The result also reveal associations between age and the prevalence of abnormal semen parameters. While the majority of participants were in the age group of 25-30 years, a higher percentage of abnormal semen samples were observed in this age group compared to others. This underscores the importance of age as a potential factor influencing male fertility. Additionally, the duration of infertility appears to have a correlation with semen abnormalities, with a higher proportion of abnormal semen parameters observed in couple experiencing longer duration of infertility.

Severity of semen abnormalities: The severity of semen abnormalities observed in the study is notable with a considerable proportion of participants exhibiting severe forms of oligozoospermia, asthenospermia, and other abnormalities. This highlights the significant reproductive challenges faced by male partners of infertile couples and underscore the importance of comprehensive evaluation and management strategies in such cases.

Normozoospermia: The recent study reports a normozoospermia rate of 36%, which falls between the rates reported by Aulia et al. (50.3%) and Jairajpuri et al. (33%).It's lower compared to Kurdukar et al. (55%) and higher than Onyebuchi et al. (16%) and Temesgen Tilahun et al. (16%).

Oligozoospermia is the most common cause of male infertility.:The recent study has an oligozoospermia rate of 57.3%, out of which severe oligospermia observed in 10.2 % of the analyzed sample, which is higher than all the other studies except Kurdukar et al. (30%).Onyebuchi et al. (48.9%) and Aulia et al. (38.6%) report lower rates compared to the recent study **(Table 6).**

Azoospermia: The recent study reports an azoospermia rate of 16.2%, which is lower than Onyebuchi et al. (24.4%) and Jairajpuri et al. (24.4%).t's higher than Aulia et al. (11.7%) and Kurdukar et al. (10%). The abnormalities observed in sperm parameters could potentially be attributed to dysfunction in the hypothalamic-pituitary-testicular axis, blockages within the male reproductive tract, or impaired sperm production.[12-14] Considering these factors, the authors suggest conducting testicular biopsy, hormonal analysis, and chromosomal studies in such male partners of infertile couples to gain deeper insights into the underlying causes of infertility.[13,15] **(Table 6).**

Evaluating sperm cell movement is crucial because sperm need to navigate through the cervical mucus and traverse the female reproductive tract to reach and fertilize the egg in the fallopian tube.[5,16] Additionally, motility serves as a gauge of the sperm's ability to penetrate the protective layers surrounding the egg, known as the corona radiata and zona pellucida, during the fertilization process.[5,17] In the our study, rate of asthenozoospermia 14.7%, which is lower compared to Onyebuchi et al. (43.5%) and Kurdukar et al. (27.5%).It's higher than Aulia et al. (23.4%) and Jairajpuri et al. (5.9%) **(Table 6)**.

The shape and structure of sperm cells play a significant role in male fertility. The presence of a sufficient number of normally shaped sperm cells in the ejaculate is biologically crucial. Sperm with abnormal shapes can negatively impact the fertilization rate, making sperm morphology an important factor to consider in assessing male fertility.[5,18] Teratozoospermia:The recent study reports a teratozoospermia rate of 7.4%, which is lower compared to all other studies except Jairajpuri et al. (2.6%).It's higher than Onyebuchi et al. (27.5%) and Aulia et al. (36%).Necrospermia: The recent study reports a necrospermia rate of 4.4% **(Table 6)**.

Clinical implications: The findings of this study have important clinical implication for the management of infertility. The high prevalence of semen abnormalities emphasizes the importance of semen analysis as a functional diagnostic tool in the evaluation of male infertility. Furthermore, identifying specific patterns of semen abnormlities can guide clinicians in tailoring appropriate interventions and treatment strategies to address underlying reproductive issues effectively.

Limitations and future directions: It's important to acknowledge the limitation of this study, including it's retrospective design and reliance on a single study site. Future research endeavors could benefit from prospective, multicenter studies involving larger and more diverse population to validate and expand upon these findings. Additionally, exploring the potential influence of various environmental, genetic, and lifestyle factors on semen quality and fertility outcomes would provide valuable insights into preventive and therapeutic approaches for male infertility.

V. Conclusion

In conclusion, this study contributes to our understanding of semen analysis patterns among male partners of infertile couples and highlights the high prevalence and severity of semen abnormalities in this population. In this study both sperm quantity and quality were more affected when compared to similar studies. Only 38 i.e. 36% of analyzed samples had normal semen parameters. By elicidating these findings, clinician can better tailor interventions to address male infertility and improve reproductive outcome for affected couples.

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