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Clinical Insights into Semen Parameters of Men Reporting to Two Fertility Centres in Thiruvananthapuram, Kerala, India

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Abstract

Infertility, which is defined as the failure to conceive after 12 months of regular, unprotected sexual activity, has a substantial impact on societal dynamics and frequently results in social stigma, especially against women. However, male infertility, which is defined as the inability of a male partner to impregnate a viable female, accounts for 30-40% of infertility cases. Genitourinary disorders such as varicocele, cryptorchidism, and testicular failure, and lifestyle factors like obesity, smoking, and alcohol intake are among the factors that contribute to male infertility. The purpose of this study is to investigate the types and frequency of anomalies in the sperm of 100 men who attended two reproductive clinics in Thiruvananthapuram, Kerala, India. Semen samples were collected, examined, and classified by WHO guidelines. People with leukocytospermia, reproductive tract infections, and genetic abnormalities were excluded. The most frequent abnormality was asthenozoospermia followed by oligoasthenozoospermia, whereas, teratoasthenozoospermia and oligoteratoasthenozoospermia were the least common, with no instances of isolated oligozoospermia or teratozoospermia. Considerable reductions in semen parameters were seen in certain infertility conditions when compared to normozoospermia. Sperm count, motility, and morphological defects showed moderate to strong correlations. Despite certain constraints, such as a limited sample size and exclusive emphasis on sperm analysis, the results offer a significant understanding of the subtle aspects of male infertility in the Indian subcontinent.

Keywords: Asthenozoospermia, Male Infertility, Motility, Oligoasthenozoospermia, Oligoteratoasthenozoospermia, Sperm Count, Teratoasthenozoospermia

1. Introduction

Infertility is a worldwide concern impacting both urological and gynaecological health, and it is a primary reason for gynaecological consultations[1-3]. In clinical terms, it is described as the failure to conceive a child after 12 months or more of consistent, unprotected sexual activity. Male infertility denotes the condition wherein a male partner is unable to impregnate a fertile female counterpart successfully[4]. Infertility remains a sensitive issue in society, often associated with significant social stigma[1,2]. This stigma disproportionately targets women, who are commonly held responsible for infertility, leading to numerous challenges such as economic difficulties, social isolation, marital strife, emotional turmoil, and general life dissatisfaction[1]. The recognition of the male contribution to infertility has gained prominence only during the past fifty years[5].

According to a review of 277 health surveys carried out globally, 48.5 million couples struggle with infertility[6]. The percentage of primary infertility in India varies from 3.9% to 16.8%[7]. Studies show that male factors cause 30-40% of cases of infertility, and female factors cause 30-40%. Furthermore, a significant portion of instances (20-40%) either combine

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the two elements or remain unexplained[8]. It has been determined that diseases affecting several body systems have a major role in male infertility[9]. Factors including varicocele, cryptorchidism testicular failure, etc., fall within the category of genitourinary illnesses and are associated with male infertility[10,11]. Additionally, it has been demonstrated that some lifestyle choices, such as obesity, smoking, alcohol consumption, using recreational drugs, being exposed to radiation, etc., negatively affect the number and quality of sperm. At the microscopic level, these factors could show up as oligozoospermia (low sperm count), asthenozoospermia (reduced sperm motility), necrozoospermia (immobile or deceased sperm), azoospermia (lack of sperm in the ejaculate), teratozoospermia (abnormal sperm morphology), or a combination thereof[10].

Semen analysis persists as a steadfast method for evaluating male factor infertility, offering an objective assessment that is both cost-effective and accessible, typically found in the arsenal of early diagnostic tools within infertility clinics[12]. Usual sperm parameters include semen volume, viscosity, pH, motility, vitality, concentration, total sperm count, etc. These measures offer a comprehensive insight into testicular function, hormonal homeostasis, as well as the operational status of accessory reproductive organs. Assessment of these parameters can unveil the potential reasons underlying infertility, guiding the selection of suitable therapeutic interventions[13].

There is very little research undertaken in Asian countries, particularly on the Indian subcontinent in this context[14]. Hence, this study endeavours to uncover prevalent nuances in semen attributes of infertile men who visit infertility facilities in two local hospitals in Thiruvananthapuram.

2. Objectives

To study the abnormalities observed in semen parameters among infertile men at two prominent infertility clinics in Thiruvananthapuram, Kerala, India, with which our group has ongoing research programs to identify molecular defects associated with the spermatozoa in causing male factor infertility.

3. Materials and Methods

3.1 Study Area

KJK Hospital and Sree Krishna Center for Fertility, are distinguished institutions specializing in infertility and gynaecology, situated in Thiruvananthapuram, Kerala, India. These were founded with the mission to support individuals and couples facing infertility.

3.2 Subjects

The study focused on couples who attended the infertility clinic for treatment. A cohort of 100 men, of the age group of 30 years to 45 years who failed to father a child during the past year with their respective partners who were found to be clinically normal concerning their fertility were enrolled in the study. Individuals with leukocytospermia, reproductive tract infections, erectile dysfunction, sexually transmitted infections, or genetic anomalies were excluded from participation. Informed consent was obtained from these participants as per Institutional and ICMR guidelines before their enrolment in the study program.

3.3 Sample Collection and Analysis

After briefing the participants about the study goals, semen samples were obtained through self-stimulation into sterile 10 mL containers following a minimum abstinence period of three days. Subsequently, the samples were left to liquefy for 30 minutes at 37°C. Following liquefaction, the samples were diluted with 1X PBS and centrifuged at room temperature to isolate the spermatozoa. Following the guidelines and standards provided by the World Health Organisation[15], the semen analysis was carried out. Sperm count, motility, appearance, etc. were evaluated during the analysis.

This study employed the following operational definitions:

Normozoospermia: Sperm with normal parameters.

Oligoasthenozoospermia: Sperm with poor motility and low count.

Asthenozoospermia: <40% Sperm motility; <32% progressive motility.

Teratoasthenozoospermia: Sperm with poor motility and abnormal morphology.

Oligoteratoasthenozoospermia: All parameters are abnormal.

3.4 Data Analysis

Statistical analysis of data was performed using GraphPad Prism. Analysis of variance and unpaired Student's t-test was conducted to compare the semen parameters of the infertile group with those of the normozoospermic group.

Additionally, Pearson correlation analysis was used to examine the relationships between various parameters within the infertile samples.

3.5 Ethical Statement

The study has been approved by the Institute of Human Ethics Committee under reference number IHEC/01/2019/09.

4. Results

The results of semen samples were analysed based on the latest WHO[15] Guidelines. The diversity of semen sample conditions, whether occurring separately or in various combinations, is illustrated in Figure 1. According to the findings, of the total 100 samples gathered from the clinics, 58 samples were asthenozoospermic, 28 were normozoospermic, 10 were oligoasthenozoospermic, 2 were teratoasthenozoospermic, and 2 were oligoteratoasthenozoospermic (Figure 1). Notably, the majority of the samples were asthenozoospermic. It is worth mentioning that there were no instances where the sample exhibited solely teratozoospermia or oligozoospermia. However, such were occasionally present in combination with other abnormalities.

The sperm counts from the samples were analyzed and plotted against various infertility conditions, with the X-axis representing different infertility categories and the Y-axis representing sperm count (Figure 2). The data elucidate the variability in sperm counts across different infertility profiles, emphasizing the complexity of male reproductive health. Specifically, for oligoasthenozoospermia and oligoteratoasthenozoospermia, the sperm counts were observed to be below 14 million cells/mL, whereas normozoospermic conditions consistently exhibited counts exceeding 40 million cells/mL.

Figure 3 represents the distribution of normal forms of sperm in collected semen samples across various infertility conditions, with the X-axis representing different infertility conditions and the Y-axis depicting the percentage of normal forms. For teratoasthenozoospermia and oligoteratoasthenozoospermia, the incidence of normal forms was found to be less than 4%. The low percentages of normal forms of sperm highlight the gravity of abnormal sperm morphology associated with these conditions. Conversely, other samples exhibited a significantly higher percentage of normal forms.

Different motility patterns associated with each infertility condition are shown in Figure 4. In the case of asthenozoospermia, motility ranged from 7% to 38%. Conversely, oligoasthenozoospermia, teratoasthenozoospermia, and oligoteratoasthenozoospermia exhibited lower motility percentages, falling in the range of 0% to 22%, suggesting a more severe impairment in sperm motility across these specific conditions.

A two-way ANOVA was conducted to examine the associations among three distinct semen parameters concerning normozoospermic and various infertility conditions, utilizing GraphPad Prism (Figure 5). The results revealed a statistically significant decline in all semen parameters across different infertility conditions compared to normozoospermic conditions, except motility in the case of teratoasthenozoospermia.

All conditions of infertility, irrespective of their specific conditions, were consolidated and juxtaposed with normozoospermic individuals across three

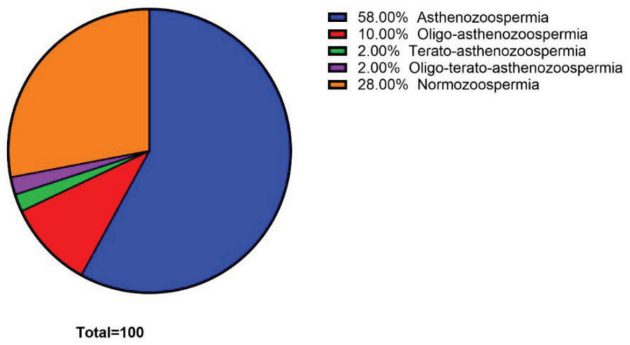


Figure 1. Different semen sample conditions in the study population.

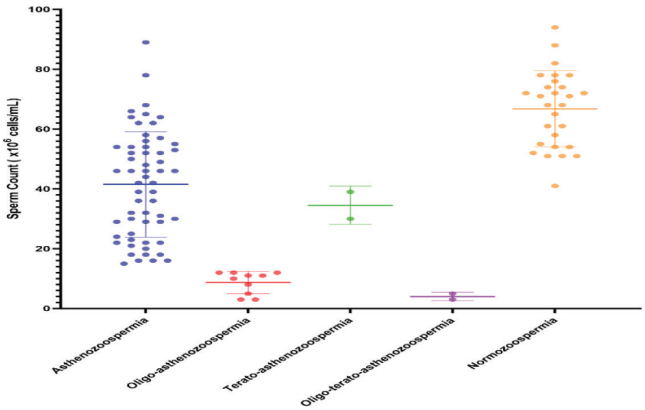


Figure 2. Graph showing sperm count in different infertility conditions.

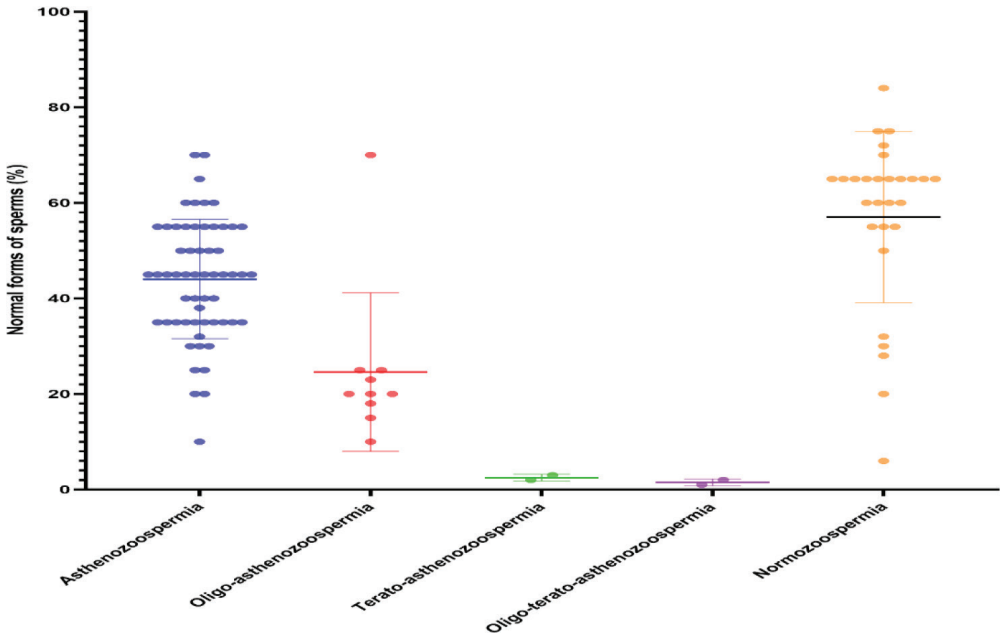


Figure 3. Graph showing normal forms of sperm in different infertility conditions.

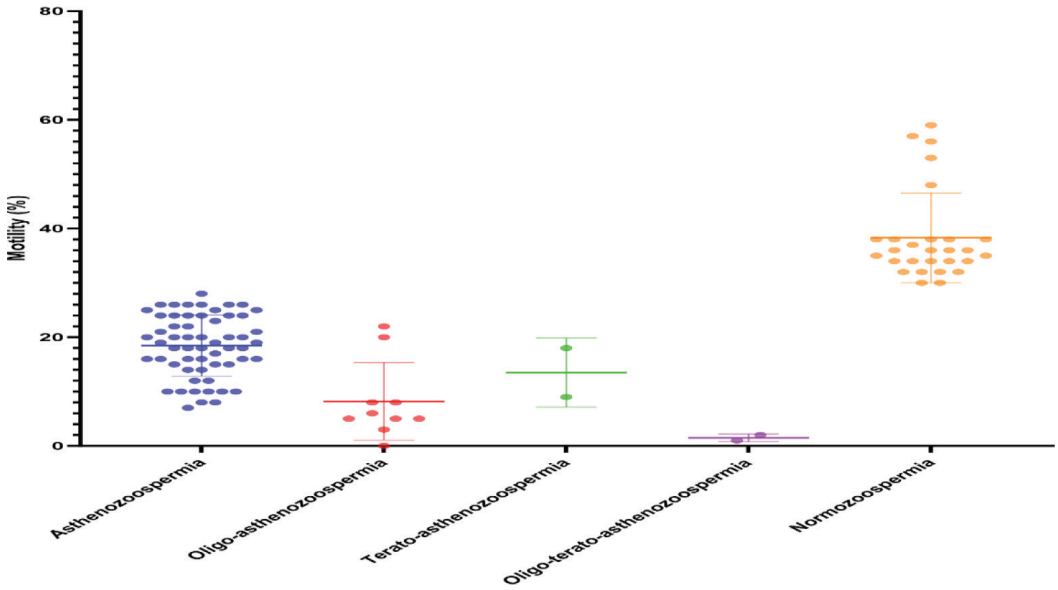


Figure 4. Graph showing sperm motility in different infertility conditions.

parameters, as illustrated in Figure 6. Utilizing GraphPad Prism, statistical analysis employing an unpaired Student's t-test unveiled a significant reduction in all assessed parameters within the infertile group compared to the normozoospermic cohort.

Pearson correlation analysis was employed to assess the relationships between various parameters in infertile samples. The results indicated a moderately negative correlation between sperm count and head defects (-0.51), motility and head defects (-0.55), normal forms and head defects (-0.59), head defects and mid-piece defects (-0.69), head defects and tail defects (-0.56), and head defects and cytoplasmic droplet defects (-0.64). Additionally, there was a significant moderate positive correlation between sperm count and motility (+0.69), as well as between sperm count and normal forms (+0.51). Notably, a high positive correlation (+0.78) was found between motility and normal forms (Figure 7).

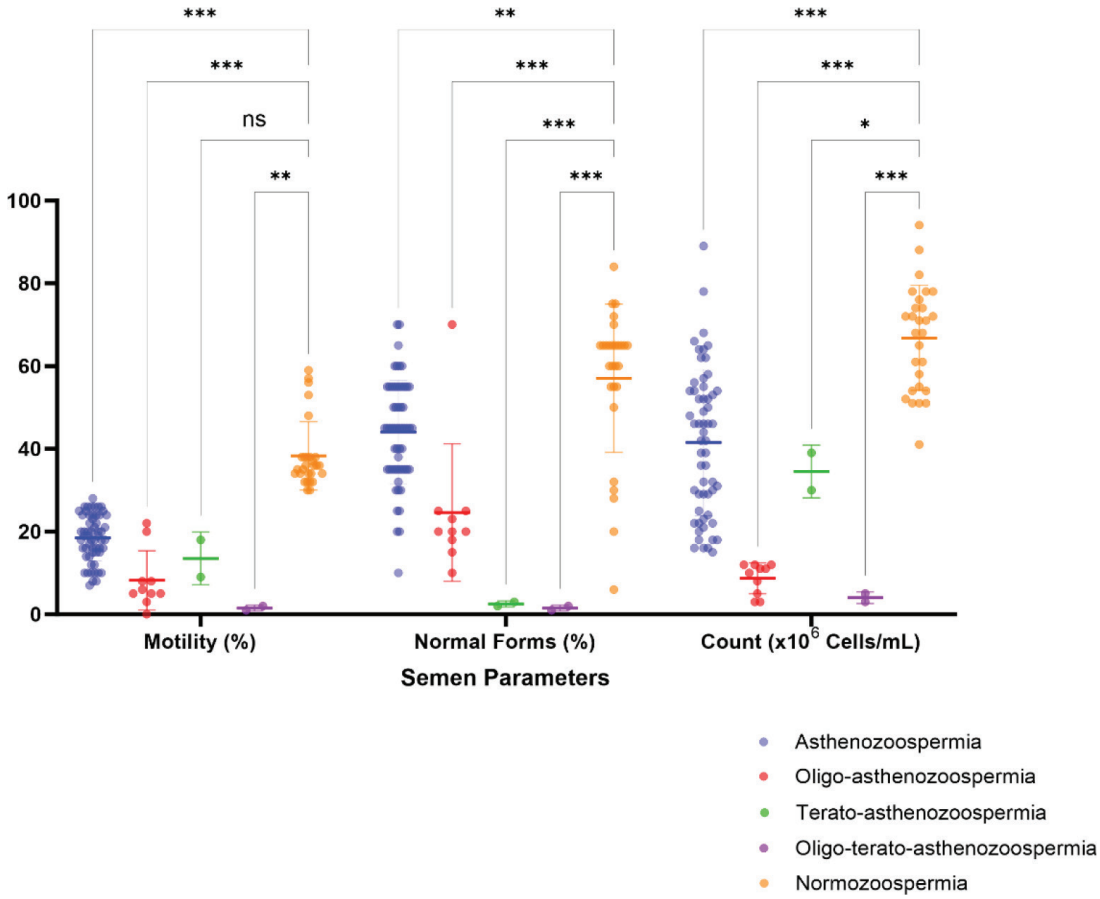


Figure 5. Graph showing associations among three distinct semen parameters concerning normozoospermic and various infertility conditions.

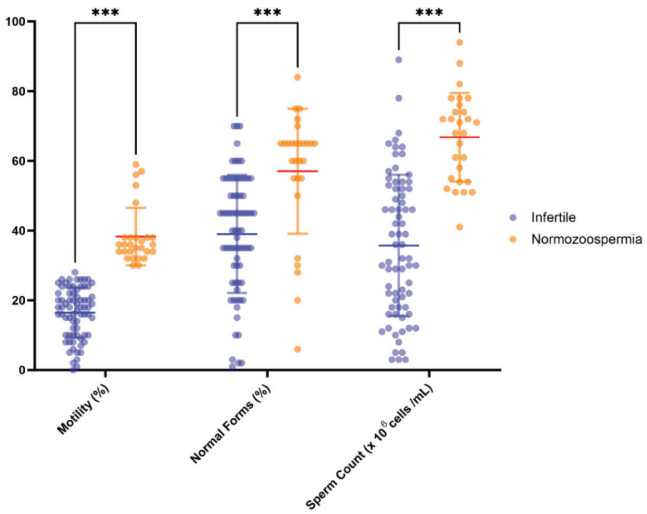


Figure 6. Graph showing the comparison of semen parameters between infertile and normozoospermic individuals.

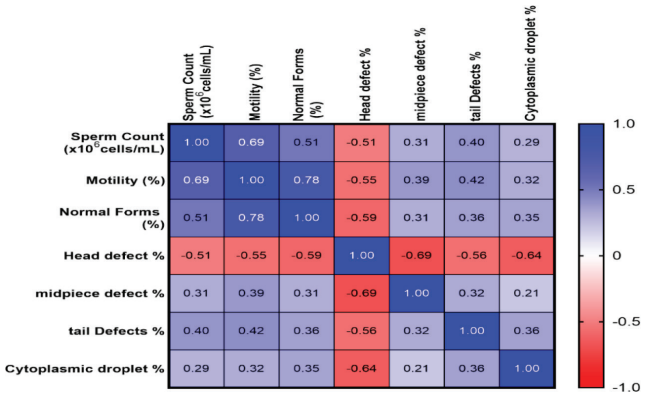


Figure 7. Heat map showing a correlation between different semen parameters of infertile samples.

5. Discussion

Male infertility, which affects a sizable percentage of men worldwide, is a major reproductive health concern. This study, which included 100 participants from two different

fertility clinics in Thiruvananthapuram, India, examined the complicated terrain of male infertility and revealed a subtle interaction of variables that highlight the intricacy of the reproductive challenges that males now deal with. In the current study, it was observed that only 28% of the participants exhibited normozoospermia, indicating normal sperm parameters. This finding contrasts with the remaining 72%, who showed signs of infertility in different conditions, including asthenozoospermia, oligoasthenozoospermia, teratoasthenozoospermia and oligoteratoasthenozoospermia. This disparity draws attention to the prevalence of male infertility as well as its various manifestations, each of which poses a different set of challenges to a successful conception.

In this study, asthenozoospermia was identified as the predominant abnormality, affecting 58% of the participants, followed by oligoasthenozoospermia at 10%. This finding aligns with the research conducted by Adenji *et al.*[1] which reported asthenozoospermia as the most frequent disorder, occurring in 27.8% of the 824 male partners studied. This trend is further supported by Racovita *et al.*[16], who reported asthenozoospermia in 30.2% of 1394 men evaluated between 2012 and 2018. The significant prevalence of asthenozoospermia in these studies is likely attributable to a high rate of genital tract infections among the affected individuals[17]. Oligoasthenozoospermia, characterized by both diminished sperm motility and a low sperm count, represents a significant concern in male fertility. This study reveals a higher incidence of oligoasthenozoospermia compared to other studies in regions, including Indonesia, where the rate is 5.7%[18], and Ebonyi State, Nigeria, with a rate of 4.3%[19]. This dual abnormality in semen parameters indicates its impact on both the quantity and quality of sperm, thus affecting fertility outcomes. According to existing literature, oligoasthenozoospermia is recognized as the most prevalent cause of male infertility[20]. This condition has a wide range of etiologies, including varicocele, cryptorchidism, metabolic problems, hormone imbalances, persistent infections, and psycho-emotional stress. Interestingly, the underlying causes are still unknown in roughly 20% of cases, although they may be related to particular genetic abnormalities[16].

There were no single cases of oligozoospermia or teratozoospermia found in this investigation. Rather, these conditions were found in conjunction with other anomalies. In particular, 2% of instances each of oligoteratoasthenozoospermia and teratoasthenozoospermia were noticed. These results are in line with earlier studies that found 2.7% of oligoteratoasthenozoospermia and a comparable frequency of teratoasthenozoospermia[21]. These conditions highlight the multifaceted nature of male infertility, where sperm count, motility and morphology play crucial roles in reproductive success.

The study findings revealed that compared to asthenozoospermia, conditions such as oligoasthenozoospermia, teratoasthenozoospermia, and oligoteratoasthenozoospermia exhibit significantly lower motility percentages. Evaluation of sperm motility is crucial because sperm must navigate through the female genital tract to fertilize the oocyte, an essential step for normal pregnancy[22]. This parameter is thus essential for determining the quality of semen and potential for conception. Sperm motility determines the efficiency with which sperm penetrate cervical mucus, traverse the female genital tract, and ultimately breach the corona radiata and zona pellucida to achieve fertilization[23]. Impaired prostate and seminal vesicle secretory processes may lead to reduced motility[24].

The current study also revealed that the incidence of normal sperm forms was below 4% in cases of teratoasthenozoospermia and oligoteratoasthenozoospermia, while other abnormal conditions demonstrated a significantly higher percentage of normal forms. Male fertility is greatly influenced by sperm morphology; the total number of morphologically normal spermatozoa in the ejaculate is physiologically significant. Unusual morphology in sperm cells has a detrimental effect on the rate of fertilisation[25]. The current study revealed that sperm counts in cases of oligoasthenozoospermia and oligoteratoasthenozoospermia were lower (< 14 million cells/mL) compared to those with normozoospermia. A decreased sperm count can serve as a potential indicator of diverse underlying health conditions or lifestyle influences[26].

The observed correlations suggest that various sperm defects are interconnected. The negative correlations between head defects and sperm count, motility, and normal forms suggest that head defects might compromise overall sperm count, health and functionality. On the other hand, the positive correlations between sperm count and motility, sperm count and normal forms, and especially between motility and normal forms, indicate that healthier sperm tend to exhibit multiple favourable traits simultaneously. The strong positive correlation

between motility and normal forms emphasizes the critical role of proper sperm morphology in enabling effective motility. Understanding these correlations is essential for diagnosing and treating male infertility, as addressing the underlying causes can improve multiple parameters simultaneously, enhancing overall fertility.

The findings of this research may exhibit variability due to regional ethnic disparities, environmental fluctuations, dietary differences, social dynamics, and evolving lifestyle patterns. The primary constraint of this study is the small sample size, potentially leading to statistical inaccuracy. It exclusively focused on sperm analysis results without investigating possible causes and risk factors. Additionally, the study was confined to two fertility clinics only, limiting the broader applicability of its findings. Furthermore, it did not account for confounding variables such as physical activity, dietary habits, nutritional intake, and overall lifestyle choices within the study population. Despite these limitations, the research encompassed subjects from diverse backgrounds, including both rural and urban areas, suggesting cautious generalizability of the findings.

6. Conclusions

The study concludes that there are notable differences in semen parameters between various infertility conditions based on the findings. The prevalence of asthenozoospermia was highest among the samples analyzed, followed by various combination anomalies. The study found that sperm counts in oligoasthenozoospermic and oligoteratoasthenozoospermic situations were much lower than in normozoospermic conditions, emphasising the severity of these abnormalities. Furthermore, the percentage of normal sperm forms was extremely low in circumstances such as teratoasthenozoospermia and oligoteratoasthenozoospermia, reinforcing the importance of aberrant sperm morphology in infertility. The observed motility patterns also varied, with substantial deficits seen in more complex infertility situations. Statistical analyses revealed substantial reductions in semen parameters across all infertile conditions when compared to normozoospermic samples. The correlation between several sperm defects revealed further information about their relationships. Overall, these findings emphasise the diverse character of sperm abnormalities in the context of infertility and the importance of extensive diagnostic evaluations to better understand and treat male infertility.

7. Acknowledgement

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8. References

1. Adeniji RA, Olayemi O, Okunlola MA, Aimakhu CO. Pattern of semen analysis of male partners of infertile couples at the University College Hospital, Ibadan. West Afr J Med. 2003; 22(3):243-5. https://doi.org/10.4314/wajm.v22i3.27959
2. Aduloju PO, Adegun PT. Factors predictive of abnormal semen parameters in male partners of couples attending the infertility clinic of a tertiary hospital in southwestern Nigeria. S Afr J Obstet Gynaecol. 2016; 22(2). https://doi.org/10.7196/SAJOG.2016.v22i2.1082
3. Owolabi AT, Fasubaa OB, Ogunniyi SO. Semen quality of male partners of infertile couples in Ile-Ife, Nigeria. Niger J Clin Pract. 2013; 16(1):37-40. https://doi.org/10.4103/1119-3077.106729
4. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization; 2010.
5. Isselbacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL. Harrisons: Principles of internal medicine. Harrisons: Principles of Internal Medicine; 1994. p. 1143.
6. Kumar N, Singh AK, Choudhari AR. Impact of age on semen parameters in male partners of infertile couples in a rural tertiary care center of central India: A cross-sectional study. Int J Reprod Biomed. 2017; 15(8):497-502. https://doi.org/10.29252/ijrm.15.8.497
7. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. PLoS Med. 2012; 9(12):e1001356. https://doi.org/10.1371/journal.pmed.1001356
8. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 2015; 8(4):191-6. https://doi.org/10.4103/0974-1208.170370
9. Katz DJ, Teloken P, Shoshany O. Male infertility - The other side of the equation. Aust Fam Physician. 2017; 46(9):641-6.
10. Sharma A. Male infertility; evidences, risk factors, causes, diagnosis and management in human. Ann Clin

Lab Sci. 2017; 05(03). https://doi.org/10.21767/2386-5180.1000188

11. Bayasgalan G, Naranbat D, Radnaabazar J, Lhagvasuren T, Rowe PJ. Male infertility: Risk factors in Mongolian men. Asian J Androl. 2004; 6(4):305-11.
12. Patel AS, Leong JY, Ramasamy R. Prediction of male infertility by the World Health Organization laboratory manual for assessment of semen analysis: A systematic review. Arab J Urol. 2018; 16(1):96-102. https://doi.org/10.1016/j.aju.2017.10.005
13. Juneja P, Phukan PK, Changmai D. A study of abnormal semen parameters in infertile couples in Assam, India. Int J Reprod Contracept Obstet Gynecol. 2019; 8(3). https://doi.org/10.18203/2320-1770.ijrcog20190870
14. Asif M, Vijay AS, Maheshwari, Fyzullah S, Rani U, Swathi R, *et al*. Impact of chronological ageing on semen parameters in southern Indian men visiting infertility centre: A retrospective study. Asian Pac J Reprod. 2023; 12:10-5. https://doi.org/10.4103/2305-0500.365227
15. Organization WH. WHO laboratory manual for the examination and processing of human semen: World Health Organization; 2021.
16. Racovita S, Sprincean M, Poneatenco D, Gorduza EV, Mosin VJOH, Management R. Development of semen quality in male partners of infertile couples in the Republic of Moldova. One Health Risk Manag. 2020; 1(1):36-41. https://doi.org/10.38045/ohrm.2020.1.06
17. Comhaire FH, Mahmoud AM, Depuydt CE, Zalata AA, Christophe AB. Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: The andrologist's viewpoint. Hum Reprod Update. 1999; 5(5):393-8. https://doi.org/10.1093/humupd/5.5.393
18. Aulia SN, Lestari SW, Pratama G, Harzief AK, Sumapraja K, Hestiantoro A, *et al*. The pattern of abnormalities on sperm analysis: A study of 1186 infertile male in Yasmin IVF clinic Jakarta. J Phys Conf Ser. 2017; 884(1):012138. https://doi.org/10.1088/1742-6596/884/1/012138
19. Onyebuchi A, Ekwunife C, Mamah J, Iwe B, Afogu E, Obi V. Seminal fluid analysis of male partners of infertile couples in Abakaliki, Ebonyi State, Nigeria. Trop J Obstet Gynaecol. 2018; 35(3). https://doi.org/10.4103/TJOG.TJOG_56_18
20. Punab M, Poolamets O, Paju P, Vihljajev V, Pomm K, Ladva R, *et al*. Causes of male infertility: A 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Hum Reprod (Oxford, England). 2017; 32(1):18-31. https://doi.org/10.1093/humrep/dew284
21. Sharique AA, Sharique M, Begum S, Shah SW. Insight into male infertility: Assessment of pattern of semen abnormalities. Ann. Abbasi Shaheed Hosp Karachi. 2016; 21(3):147-53.
22. Butt F, Akram N. Semen analysis parameters: Experiences and insight into male infertility at a tertiary care hospital in Punjab. JPMA The J Pak Med Assoc. 2013; 63(5):558-62.
23. Jairajpuri ZS, Rana S, Ali MA, Pujani M, Jetley S. Patterns of semen analysis: Experiences of a laboratory catering to semi urban population of Delhi. Bangladesh J Med Sci. 2017; 16(2):314-19. https://doi.org/10.3329/bjms.v16i2.31944
24. Verze P, Cai T, Lorenzetti S. The role of the prostate in male fertility, health and disease. Nat Rev Urol. 2016; 13(7):379-86. https://doi.org/10.1038/nrurol.2016.89
25. Homa ST, Vessey W, Perez-Miranda A, Riyait T, Agarwal A. Reactive Oxygen Species (ROS) in human semen: Determination of a reference range. J Assist Reprod Genet. 2015; 32(5):757-64. https://doi.org/10.1007/s10815-015-0454-x
26. Ferlin A, Garolla A, Ghezzi M, Sartori L, Plebani M, Foresta C. Sperm count and hypogonadism as markers of general male health. Eur Urol Focus. 2021; 7(1):205-13. https://doi.org/10.1016/j.euf.2019.08.001