Original Article

Patterns of Semen Analysis in Male Partners of Infertile Couples at a Tertiary Care Centre of Eastern India

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ABSTRACT

Introduction: The failure to obtain a clinical pregnancy after 12 months or more of regular, unprotected sexual contact defines infertility, a disease of the reproductive system. It affects demographics, the economy, psychology, and health. Semen analysis is frequently used in infertile relationships to assess the male partner. An abnormal set of semen parameters and a male-infertility-related factor are discovered in 50% of partners who are unintentionally childless. Information on male infertility and semen research is scarce in India.

Aims/ objective: To determine the pattern of semen analysis results in male partners of infertile couples at tertiary care centre of eastern India.

Materials and Method: After the proper dilution, the hemacytometer was used to quantify the sperm. Under a microscope, motility was seen in a wet sample. The Eosin-Nigrosin stain was used to determine the viability of spermatozoa by detecting those with intact membranes. The semen sample was centrifuged, and smears were made and stained with Papanicoloau and haematoxylin and eosin stains for the purpose of evaluation of morphology. Semen analysis parameters were categorised using lower reference levels provided by the WHO for semen analyses.

Results: More than 90% of cases had normal semen volume and more than 80% of them had normal pH. Nearly 50% of the cases had low sperm cells and percentage of vital sperm cells was inadequate in 66.95% of the cases. Only 16.95% of male partners were having semen with low pH whereas viscosity was high in nearly 30% of the cases. Most common abnormality was Asthenozoospermia (reduced sperm cell motility) with prevalence of 43.22% followed by Teratozoospermia (increased abnormal forms of sperm) (27.12%).

Conclusion: Reduced sperm cell motility was discovered to be the most frequent cause of male infertility in our research. Our research also showed that as age of person increases, their sperm counts tend to decline. It is strongly advised that advanced diagnostic modalities be introduced and risk factors for male infertility in the study region be identified.

Keywords: Semen Analysis, Male Infertility, Sperm Count, Sperm Motility, Sperm Morphology.

INTRODUCTION

The failure to obtain a clinical pregnancy after 12 months or more of regular, unprotected sexual contact defines infertility, a disease of the reproductive system.[1] It affects demographics, the economy, psychology, and health. Because it affects a couple rather than just one person, infertility is a threat to health of whole family.

There can be primary or secondary infertility. Women who have not previously given birth are considered to have primary infertility. There is at least one pregnancy but no subsequent conceptions in secondary infertility. [2, 4 –6]

Although infertility is a worldwide problem, the precise frequency is unknown. It occurs more frequently in underdeveloped nations. According to reports from various nations around the globe, it typically ranges from 5% to 30%. [2,7] According to the World Health Organization, 60–80 million couples globally struggle with infertility. [8, 9] The burden of infertility in India has not yet been thoroughly researched. [10]

The male or female, or both, may be the etiologic source of infertility. [2, 11] Male and female variables each make up 30%–40% of the total contribution, while in 35% of cases, both partners have reproductive disorders. Only 5% of instances of infertility are unsolved. [12 –14] Congenital or acquired urogenital abnormalities, cancers, urogenital tract infections, elevated scrotal temperature, endocrine disorders, genetic abnormalities, sexual dysfunction, ejaculatory issues, and immunological variables can all affect male fertility. [9, 11, 13]

Male infertility is the incapacity of a man to result in conception in a fertile woman. Semen analysis is frequently used in infertile relationships to assess the male partner. An abnormal set of semen parameters and a male-infertility-related factor are discovered in 50% of partners who are unintentionally childless. [14]

Male infertility treatment is complicated and requires a methodical strategy. Semen research is one of the most commonly used investigations. It is still used as a helpful investigation for infertile couples and is the typical first-line test for assessing male infertility. [6, 13, 15] The functional capacity of the spermatozoa to fertilise ova is determined in large part by the semen parameters. [13] Semen analysis reveals observable abnormalities in males who are infertile. They have abnormal morphology, insufficient sperm concentration, and/or weak sperm motility. As a result, a thorough analysis of the semen parameters may reveal some potential reasons for male infertility. [8, 11]

Information on male infertility and semen research is scarce in India. Therefore, this study was done to determine the pattern of semen analysis results in male partners of infertile couples at tertiary care centre of eastern India.

MATERIALS AND METHODS

This was a retrospective study done in tertiary care centre of eastern India in which laboratory reports of male partner of infertile couples visiting out-patient department of obstetrics & gynaecology from August 2020 to July 2021 was assessed. The study was conducted after approval of institutional ethics committee and waiver of informed consent was taken due to retrospective design of study.

Inclusion Criteria: The research included all male partners who underwent semen analysis while receiving treatment for infertility at the out-patient department of obstetrics and gynaecology during the study period.

Exclusion Criteria: Exclusion criteria for the research included cases with incomplete records, men with a history of drug use, men who had fever within the previous six months, chronic illnesses like diabetes mellitus, hypertension, endocrine diseases, and exposure to chemotherapeutic or radiotherapy agents.

Laboratory Procedure: The couples were told to refrain from sexual activity for three to seven days. At a particular sample collection room, samples were taken by masturbation or coitus interruptus into a sterile plastic container. After being collected, all samples were kept for 30 to 1 hour at 37 °C before being examined. The World Health Organization (WHO) laboratory manual for the inspection and processing of human semen 2010 was adhered to in terms of procedures and standards. After the proper dilution, the haemocytometer was used to quantify the sperm. Under a microscope, motility was seen in a wet sample. Using the Eosin-Nigrosin stain, a vitality test was performed by detecting spermatozoa with intact membranes. The number of dead (stained) and live (unstained) spermatozoa were then counted. The semen sample was centrifuged, and smears were made and stained with Papanicoloau and haematoxylin and eosin stains for the purpose of evaluation of morphology. In order to compare the pH value with a calibration strip, pH paper was used to determine the power of hydrogen (pH) value.

Assessment: The WHO updated lower reference levels for semen analyses in 2010. [5] The accepted 5th percentile (lower reference boundaries) and 95% confidence intervals (CIs) are represented by the following parameters:

Semen volume: 1.5 mL (95% CI: 1.4 to 1.7); Sperm concentration: 15 million spermatozoa/mL (95% CI : 12 to 16); Morphology: 4% normal forms (95% CI : 3 to 4); Vitality: 58% live spermatozoa (95% CI : 55 to 63); Progressive motility: 32% (95% CI : 31 to 34); and Total motility (progressive plus non-progressive motility): 40% (95% CI : 38 to 42). [6]

The following terms were used in this study:

- **Normozoospermia:** All semen parameters are normal
- **Severe oligozoospermia:** Sperm cell count per ml < 5 million
- **Asthenozoospermia:** Reduced sperm cell motility
- **Azoospermia:** No sperm cells in semen
- **Necrozoospermia:** All sperm cells are non-viable
- **Teratozoospermia:** Increased abnormal forms of sperm

Statistical Analysis: The data were represented in the tabular form using Microsoft excel 365 and then transferred into graph pad version 8.4.3. Descriptive analysis was done to obtain and compare frequency, percentage, and average of the data.

OBSERVATIONS AND RESULTS

The patient's medical records and laboratory register of the hospital were reviewed to get 127 semen samples which were analysed from August 2020 to July 2021. We included all cases with complete documentation. Accordingly, 118 samples were included in this study.

Table 1: Age distribution of the male partners

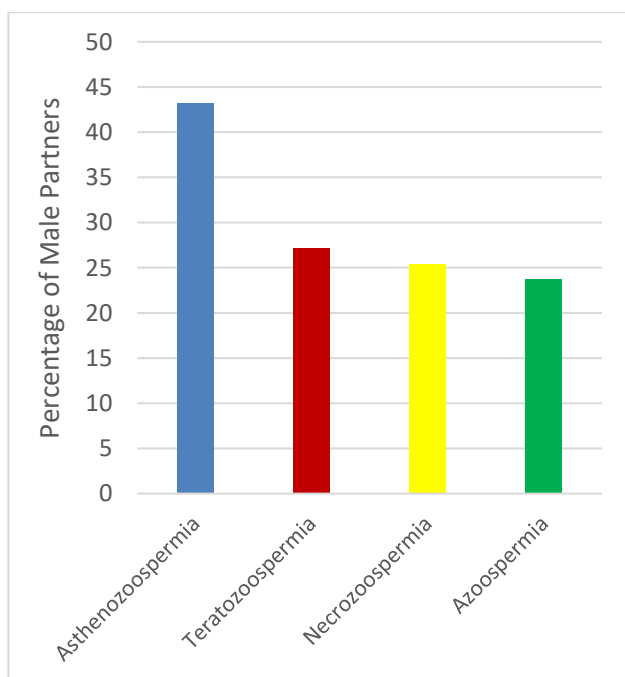| Age in Years	Number of Male Partners	Percentage, % (n = 131)
21-25	29	24.58
26-30	48	40.68
31-35	9	7.63
36-40	11	9.32
>40	21	17.80

Most of the male partners who visited for work-up of infertility were of age group of 26-30 years.

Table 2: Evaluation of different parameters of semen analysis

Sperm Parameters	Categories	Number of Male Partners	Percentage, % (n = 131)
Semen volume (ml/ejaculate)	< 1.5	10	8.47
	≥ 1.5	108	91.53
Viscosity	High	37	31.36
	Normal	81	68.64
pH of semen	< 7.2	20	16.95
	≥ 7.2	98	83.05
Sperm count (x 10^6/ml)	< 15	57	48.31
	≥ 15	61	51.69
Percentage of vital sperm cells	< 58 %	79	66.95
	≥ 58 %	39	33.05
Sperm cell motility	< 40 %	51	43.22
	≥ 40 %	67	56.78
Sperm cell morphology	< 4 %	32	27.12
	≥ 4 %	86	72.88

More than 90% of cases had normal semen volume and more than 80% of them had normal pH. Nearly 50% of the cases had low sperm cells and percentage of vital sperm cells was inadequate in 66.95% of the cases. Only 56.78% of male partners had normal sperm cell motility however sperm cells morphology was adequate in 72.88% of the cases. Only 16.95% of male partners were having semen with low pH whereas viscosity was high in nearly 30% of the cases.

**Figure 1:** Distribution of various abnormalities among male partners

Table 3: Frequency of various abnormalities among male partners

Abnormalities	Number of Male Partners	Percentage, % (n = 131)
Asthenozoospermia	51	43.22
Teratozoospermia	32	27.12
Necrozoospermia	30	25.42
Azoospermia	28	23.73

Most common abnormality was Asthenozoospermia (reduced sperm cell motility) with prevalence of 43.22% followed by Teratozoospermia (increased abnormal forms of sperm) (27.12%).

Table 4: Age-wise trends of average sperm cell counts among male partners

Age in Years	Average sperm count (x 10^6/ml)
21-25	81.29
26-30	42.88
31-35	19.21
36-40	35.37
>40	14.12
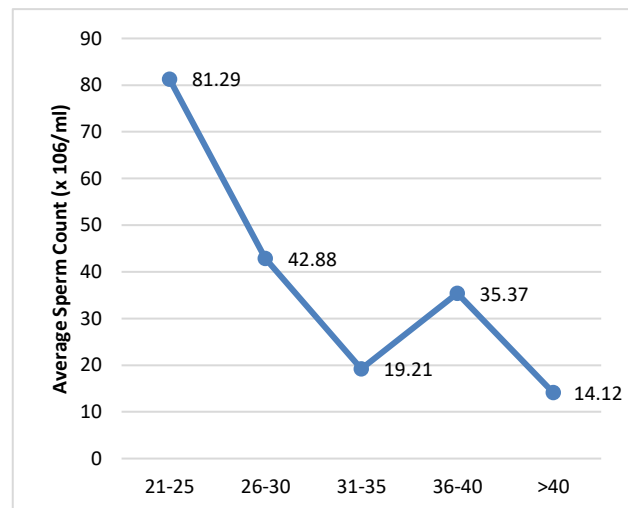
Figure 2: Age-wise trends of average sperm cell counts among male partners

DISCUSSION

This research described the tertiary care centre's semen analysis parameters in eastern India. Participants in the research ranged in age from 20 to 65, with a mean age of 29.97 + 7.92 years. In this research, one or more abnormal semen analysis parameters were present in more than 80% of the analysed samples. The absence of or a deficiency in sperm cells in the semen, as well as sperm cell motility issues, abnormal sperm cell morphology, and/or a combination of these, are the most frequent findings in this research. Compared to other research, the abnormality in these semen parameters is higher.[8,11,12,16,17]

134

This result is comparable to one from a Delhi research (84%).[18] It's possible that environmental, nutritional, socioeconomic, hormonal, genetic, and/or other factors contributed to the decline in semen quality in this research.

It is frequently suggested that sperm concentration can be used to forecast future fertility. [6] Male sterility is most frequently caused by oligospermia. 3 In this research, 48.31% of the examined samples had sperm counts that were below the WHO-established reference level. Of the samples analysed, azoospermia was found in 23.73% of the cases. It is lower than a study done in Nepal (28.8%) and similar to one done in Indonesia (24.4%). [9, 16] In comparison to studies conducted in Nigeria (11.7%), Ethiopia (14.5%), Delhi (9%), and India (10%), higher prevalence of azoospermia was found in our study. [8, 17 –19]

The variation in the study group could be the cause of the difference. This abnormal parameter could be a result of sperm cell dysfunction, obstruction of the male reproductive system, or failure of the hypothalamic-pituitary-testicular axis.[9, 14, 16] Therefore, the authors advise these male partners of infertile couples to undergo a testicular biopsy, hormonal analysis, and chromosomal analysis. [14, 20]

As the spermatozoa must engage with cervical mucus and move through the female genital tract to fertilise the oocyte in the uterine tube, measuring sperm cell motility is crucial. [6, 21] Motility is a sign of how sperm cells enter the zona pellucida and corona radiata prior to fertilisation of the ovum. [6, 18] 43.22% of the samples used in this research had total sperm cell motility below the WHO reference level. This abnormality is greater than research done in Indonesia (5.9%), Nigeria (23.4%), Delhi (22.1%), Gujarat (31.4%), and South India (27.5%).[8, 16 –18, 22]

Male fertility is also greatly influenced by the morphology of sperm cells. It is important from a biological perspective to determine the proportion of spermatozoa with normal morphology. Unusual cell shape negatively impacts the rate of fertilisation.[6, 23] 72.88% of the samples analysed in this research had normal morphology. This is less than the research done in Nigeria (64%), South India (91.4%), and Gujarat (91.4%). This finding is similar to other studies done in Nigeria (73.1%). [13, 17, 22]

In this research, triads of abnormalities in sperm concentration, motility, and morphology were found in one-fourth of the analysed samples. Oligo-asthenoteratozoospermia is the name given to this. This result is greater than that of studies conducted in Nigeria (4.3%) and Indonesia (5.7%). [16, 17] This anomaly suggests that parameters used in semen analysis, both in terms of amount and quality, were impacted.

In this research, the effects of ageing on seminal parameters were also examined. It was found that average total motility, morphology, and vitality showed an increase in their average values up to 31–34 years of age and then a sharp decline as people aged. The other two researches lend credence to this conclusion. [12, 23] The decrease in motility with ageing may be caused by rising seminal reactive oxygen species (ROS) levels as well as modifications to the function of the accessory sex glands and the epididymis.[11, 23]

Our study had certain limitations. The cause-and-effect relationship was not assessed in our study. The other drawback could be a small sample size that might result in statistical imprecision.

CONCLUSION

Reduced sperm cell motility was discovered to be the most frequent cause of male infertility in our research. Our research also showed that as age of person increase, their sperm counts tend to decline. Nearly 90% of infertile couples' male spouses had one or more abnormal semen analysis results. In comparison to previous studies, there was a greater impact on sperm quantity and quality. Given this result, it is strongly advised to identify risk factors and implement cutting-edge diagnostic modalities for the investigation of male infertility.

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