Original Article

Semen Analysis Parameters: insight into Male Infertility in a Tertiary Care Hospital

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Abstract:

Male infertility is commonly due to deficient sperm count in semen and semen quality. Semen analysis as an integral part of infertility investigations and is taken as a surrogate measure for male fecundity. This descriptive cross-sectional survey was carried out at the Microbiology Laboratory, Popular Diagnostic Centre Ltd from July 2017 to December 2017 to determine the prevalence of azoospermia, oligospermia in male infertile population, and to assess the distribution of abnormal semen parameters in infertile men. A total of 300 semen analysis was performed according to methods and standards defined by the World Health Organization (WHO), 2010. Normozoospermia was observed in 186 (62%) males, oligospermia in 81 (27%) and azoospermia in 33 (11%) males. Asthenospermia was observed in 113 (37.6%) and teratozoospermia in 11 (3.6%) of samples. Normospermia was observed in 282 males (94%), hypospermia in 18 (6%) and no male was hyperspermic. Abnormal viscosity was found in 3 males, 1 in each category. Only azoospermic (N=33) category had 4 (12.12%) male having pH <7.2. Pus cell (>5HPF) was observed in 16 (8.60%) males of normozoospermic, 15 (18.52%) males of oligospermic and 08 (24.24%) males of azoospermic category. The oligospermic samples had higher percentage (93.83%) of non-motile sperms and abnormal morphology in 13.58% in comparison to normozoospermic samples. Semen analysis is the cornerstone for the evaluation of infertility in men. Not only sperm concentration but also the motility, morphology and other seminal markers are important factor that also may have negative impact on fertility.

Key words: *Infertility; Azoospermia; Semen analysis, Sperm.*

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Introduction:

Infertility, defined as the inability of a sexually active, non-contracepting couple to conceive after 1 year of regular intercourse, is a grave concern that affecting couples causing considerable psychological distress to them.[1] Approximately about 10-15% of couples suffers from infertility all over the world. Female factor is responsible in 35% and male factor in 45% of cases while the rest of the couples either have combination of factors or unexplained infertility.[2] The exact prevalence of infertility in Bangladeshis is difficult to ascertain.[3] The rising level of male infertility has become a serious concern/problem for public health. "Male factor" infertility is seen as an alteration in sperm concentration and/or motility and/or morphology in at least one sample of two sperm analyzes, collected 1 and 4 weeks apart.[4] It is commonly due to deficiencies in semen, and semen quality is used as surrogate measure of male fecundity.[5]

Males with sperm parameters below the WHO normal values are considered to have male factor infertility.[6] The most significant of these are low sperm concentration (oligospermia),

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poor sperm motility (asthenozoospermia), and abnormal sperm morphology (teratozoospermia).[5] Other factors less well associated with infertility include semen volume and other seminal markers of epididymal, prostatic, and seminal vesicle function.[7] As high as 90% of male infertility problems are related to count and there is a positive association between the abnormal semen parameters and sperm count.[8] The problem with sperm count, motility, and morphology stems from disarray in control mechanism, including pre-testicular, testicular, and post-testicular factors.[9] The efficient passage of spermatozoa through the cervical mucus depends on rapid progressive motility[10] that is, spermatozoa with a forward progression of at least 25 ¼m/s. A normal semen analysis must contain 32% or more progressively motile spermatozoa. Persistent poor motility is a predictor of failure in fertilization.[11] Morphology should be used along with other parameters, and not as an isolated parameter when determining clinical implications.[12,13]

The infectious process in the genitourinary tract may play a contributing role in the reproductive function and fertility in males. Infectious etiologies may be involved in up to 15% of male infertility cases.[14] Although small numbers of WBCs are a normal constituent of the semen, patients are only considered non-pyospermic as long as the WBC concentration remains below 5 per high power field (HPF).[15] In particular, leukocytospermia is an abnormal laboratory finding defined by the World Health Organization as the presence of 1×10^6 leukocytes/mL in human ejaculate and reflects the presence of genital tract infection.[13]

Possible consequences of infections are impairment of the spermatogenesis, the induction of an autoimmune mechanism, spematodysfunction, and inflammatory occlusion of the ejaculatory duct.[16] Reduced motility of spermatozoa has been found in semen samples which contain high concentrations of bacteria.[17] Similarly, 43% of pyospermic patients showed spontaneous downward variation in the absence of treatment.[18] The prevalence of antisperm antibodies in the infertile population is approximately 10%. Earlier studies found that a higher prevalence of antisperm antibodies among infertile men with a history of bacterial prostitits or urethritis were pyospermic.[19] Moreover, the sperm motile index (SMI) was found to be significantly lower in pyospermic patients when compared with that of non-pyospermic men.[15] A different way to express sperm quality is the calculation of the total Motile sperm count (TMSC), which is obtained by multiplying the volume of the ejaculate in milliliters by the sperm concentration and the proportion of A (fast forward progressive) and B (slow progressive) motile sperms divided by 100%.[20] The sperm parameter morphology is not taken into account in this calculation. Another measure is FSC (Functional Sperm Concentration, M/ml) which is the concentration of progressively motile spermatozoa with normal morphology in a semen sample. Total Functional Sperm which accounts for the sample volume and report a result that represents the entire ejaculate.[21]

Hence, semen analysis remains the single most useful and fundamental investigation in the search for the cause of male infertility. It is a simple test that assesses the formation and maturity of sperm as well as how the sperm interacts with the seminal fluid so it provides insight not only on sperm production (count), but sperm quality (motility, morphology) as well.[22] Semen analysis is the investigation with a sensitivity of 89.6%, that it is able to detect 9 out of 10 men with a genuine problem of male infertility.[23] Regarding this issue, a few studies have been done in our country in small scale but this does not reflect actual scenario. The present study aimed at finding the prevalence of abnormalities of seminal parameters in infertile male population over a period of six months in a tertiary care hospital of Dhaka.

Material and methods:

This descriptive cross sectional study was carried out at the Microbiology Laboratory, Popular Diagnostic Centre Ltd, Dhanmondi over a six months period, from July to December, 2017. The sample population was the male partners of infertile couples referred for the semen analysis to the Microbiology laboratory.

A total of 300 semen samples, 50 samples in each month were collected randomly during the period of July 2017 to December 2017. The semen samples were analyzed with collaboration of laboratory technician under supervision of laboratory consultant. Semen analysis was performed by Automated Sperm Quality Analyzer IICP (BioGen, Istanbul / Turkey) according to methods and standards defined by the World Health Organization (WHO) 2010 guideline.[13]

Parameters outlined included: Appearance: grey/opalescent; Volume: 1.5 ml or more; liquefaction: <60 minutes; Viscosity: not more than 2 cm (thread length after liquefaction); pH: >7.2; Sperm concentration: >15 million/ml; Total sperm count: 39 million, per ejaculate or more; Progressive Motility: 32% or more; Morphology: 4% or more with normal form; Motile sperm concentration > 4.8 million/ml; Total functional sperm concentration (million/ml): 0-1=poor, 1.10-2=medium, >2=good; Sperm motility index (million/ml): 0-40=poor, 41-50=medium,>50=good.

Samples were categorised into normozoospermia, oligospermia and azoospermia on the basis of sperm concentration and were also categorised on basis of of motility, morphology, volume. After exclusion of azoospermic samples, normozoospermic and oligospermic samples were compared for motility and morphology. The operational definitions were: Normozoospermia: sperm count more than 15 million/ml; Oligospermia: sperm count below 15 million/ml; Azoospermia: absence of spermatozoa in the ejaculation; Astheno-spermia: reduced sperm motility; Terato-zoospermia: abnormal sperm morphology; normospermia: volume 1.5-6ml Hypospermia: volume <1.5ml; and Hyperspermia: volume >6ml.

The information collected was reviewed and inconsistencies was investigated and clarified. After editing and coding, test results and data was entered in SPSS program version 20. Frequency and 95% Confidence interval was calculated for proportions.

Results:

Among the 300 males, the mean age was 33.35± 5.55 years. Using WHO standard for semen normality, 300 samples that were analyzed, normal sperm count (normozoospermia) was observed in 186 males (62%), oligospermia in 81 (27%) and azoospermia in 33 males (11%) (Table-1). Asthenospermia was observed in 113 (37.6%) and teratospermia in 11 (3.6%) of total samples (Table-2). On volume distribution, normospermia was observed in 282 males (94%), hypospermia in 18 (6%) and no male was hyperspermic (Table-3).

Table-4 showed the frequency of semen viscosity and pH in three categories of normozoospermia, oligospermia and azoospermia. Abnormal viscosity was found in 3 males, 1 in each category. Among 300 males, only azoospermic (N=33) category had 4 (12.12%) male having pH <7.2.

Table-5 showed the frequency of pus cell and RBC. The samples that were analyzed, pus cell was observed in 16 (8.60%) males of normozoospermic, 15 (18.52%) males of oligospermic and 08 (24.24%) males of azoospermic category.

After excluding 33 samples with azoospermia, semen parameters were compared in oligospermic and normozoospermic samples for motility and morphology (Table-6). Normal motility was observed only in 154 (82.79%) of normozoospermic males. The oligospermic samples had higher percentage of low motile sperms (93.83%) compared to normozoospermia in which lowmotile sperms was 17.20%. Normal morphology of sperms was observed in 186 (100%) of normozoospermic in comparison to 70 (86.42%) of oligospermic samples. Abnormal morphology in 11 males (13.58%) and non motile sperm in 5 males (6.17%) was only observed in oligospermic males.

Table-7 showed the frequency of motile sperm concentration, total functional sperm concentration and sperm motility index between normozoospermic and oligospermic male. Oligospermic male had higher percentage of abnormal motile sperm concentration 81 (100%) than in normozoospermic male 14 (7.53%). Though in total functional sperm concentration

Table-V

Frequency of pus cell and RBC in semen

Category	Pus cell Frequency (%) N=300		RBC Frequency (%) N=300	
	0-5/HPF	>5/HPF	Present	Absent
Normozoospermia N=186	170 (99.40%)	16 (8.60%)	00 (100%)	186
Oligospermia N=81	66 (81.48%)	15 (18.52%)	00 (100%)	81
Azoospermia N=33	25 (75.76%)	08 (24.24%)	01 (3.03%)	32 (96.96%)

Table-VI

Comparison of sperm motility and morphology between normozoospermic and oligospermic male

Category	Sperm motility Frequency (%)		Sperm morphology Frequency (%)		
	Progressive	Nonprogressive	Immotile	Normal	Abnormal
Normozoospermia	154	32	00	186	00
N=186	(82.79%)	(17.20%)	(100%)		
N=81	(93.83%)	(6.17%)	(86.42%)	(13.58%)	

Table-VII

Comparison of motile sperm concentration, total functional sperm concentration and sperm motility index between normozoospermic and oligospermic male

Category	Motile sperm concentration million/ml (%)			Total functional sperm concentration million/ml (%)			Sperm motility index million/ml (%)	
	Normal	Abnormal	Good	Medium	Poor	Good	Medium	Poor
	e"4.8	<4.8	>2	1.10-2	0-1	>2	1.10-2	0-1
Normozoo Spermia N=186	172 (92.47%)	14 (7.53%)	159 (85.48%)	23 (12.36%)	4 (2.15%)	179 (96.24%)	02 (1.07%)	5 (2.69%)
Oligo Spermia N=81	00	81 (100%)	00	9 (11.11%)	72 (88.89%)	0	11 (12.36%)	70 (2.15%)

Discussion:

The issue of human infertility is mainly considered 'infertility in woman' which is a misconception. It is uncommon for a patient to present for an infertility evaluation with an abnormal semen analysis report before an extensive female partner workup has been performed. Screening of males by semen analysis provides some insight about the underlying pathological problems occurring in the male genital tract.[24]

Azoospermia stems from a problem with sperm production or a problem with sperm transport. There are varieties of factors that may contribute to either of this causes.[25] Prevalence of azoospermia in our study population was 11%, and oligospermia in 27%. The results are comparable to a study in which the prevalence of azoospermia was 14.89% and that of oligospermia 11.11%.[25] Another study conducted by Adeniji *et al.* showed the prevalence of azoospermia 6.7% and

oligospermia to be around 18.9%.[26] One study had so far sought and found evidence that declining sperm counts were impairing conception rates.[27]

Sperm motility is a critical factor in determining semen quality and fertilizing ability. Over 85% of infertile males can actually produce sperm, but their sperms are often unable to fertilize an egg.[28] Sperm motility is a requisite for normal fertilization. Motility comes with sperm maturation in their passage through the epididymis.[29] The negative impact of a low sperm count is that it is also frequently associated with reduced sperm quality, including less motility or abnormal shape.[30] Asthenospermia is still a common cause of human male infertility although advancing techniques had somewhat overcome the problems of sperm motility in infertile couples. In our study, asthenospermia was observed in 37.67% of total samples and the results were comparable to a study in which the prevalence of asthenospermia was 18.71%.[31]

Sperm morphology is a very strong indicator of a person's bodily and thus testicular health, which is strongly reacting to bodily, physiological and environmental stresses, far more than any other organ. Morphology acts as a tool in the clinical diagnosis of a patient and also as a prognostic and predictive tool for the prediction of male fertility potential.[32]

In the present study, majority (94%) of study patients had normal semen volume, while 6% showed hypospermia and no hyperspermia was detected. The results were comparable to a study where majority (89.7%) of the patients had adequate semen volume, and only 10.3% had abnormal semen volume.[33] The results were also comparable to a study conducted in Nigeria in which majority (91%) of the patients had adequate semen volume, and only 9% had abnormal semen volume of which 7.3% had hypospermia and 1.7% had hyperspermia.[34]

In the present study, the semen pH among the patients with normal sperm concentration was not different from that among those with low sperm count. Only 4 (12.12%) male had pH <7.2 who were azoospermic. A study conducted by Harraway showed similar findings.[35] Another study demonstrated that acidic pH result in decreased sperm movement and capacitation, which could be one of the mechanisms of male infertility.[36] Seminal hyperviscosity seems to be associated with reduced sperm motility, possibly due to a 'trapping effect' that prevents normal sperm progression through the female genital tract.[37,38] Only 1% of present study patients had high seminal viscosity with normal pus cell. According to Munuce *et al.*, there is no association between seminal hyperviscosity and leukospermia or the presence of sperm antibodies.[39]

Pyospermia is one of the important causes of male infertility, but the distribution, origin, and role of pus cells in semen is still controversial. The excess indicates the presence of inflammation in the genital tract such as prostatitis,vesiculitis, orchitis, urethritis, etc.[16] In the present study, pus cell (>5 HPF) was observed in 16 (8.60%) males of normozoospermic, 15 (18.52%) males of oligospermic and 08 (24.24%) males of azoospermic category. More pus cells were observed in cases where motility and concentration was compromised. Previous studies had demonstrated that pyospermia had negative impacts on sperm function and integrity.[40,41] Studies had shown that the pathogenic bacteria, leukocytes, cytokines and reactive oxygen species (ROS) might be the primary mechanisms of infertility resulting from male accessory gland infection, and broad-spectrum treatment could reduce the density of leukocytes in semen and improve the ejaculates quality.[42] Therefore, the presence of pyospermia in normozoospermic must be considered as a male infertility factor.[16] Erythrocytes (RBC) shouldn't be normally present in semen. The presence of red blood cells in the semen may be associated with tumors, injuries of the genital organs, presence of stones in the prostate and vesiculitis.[13] In the present study RBC was found in only in 1 male who was azoospermic.

In the present study, in comparison between normozoospermic and oligospermic male, the oligospermic samples had higher percentage of

low motile sperms (93.83%) compared to normozoospermic in which low-motile sperms was 17.20%. Non motile sperm was only observed in 5 (6.17%) oligospermic males. The oligospermic samples were associated with higher percentage (13.58%) of abnormal morphology as compared to normozoospermic samples (0%) although type of abnormal morphology was not specified. The results of the present study were comparable to a study in which higher percentage (53%) of abnormal morphology and abnormal motility (60%) was observed in oligospermic males than the normozoospermic males.[43]

In the present study, sperm motility index showed that oligospermic male had higher percentage of both poor 70 (2.15%) and medium grade 11 (12.36%) than in normozoospermic male. The sperm motile efficiency index (SMEI) which indicates the rate of progressively motile sperms, was low in pyospermic males of oligospermic category. Similar findings were observed in a study by Satoh *et al.*[15] Antisperm antibodies play a significant role in male factor infertility that was not observed in present study which was one of the limitations.

Conclusion:

Male factor is important for fertility as well as female factor. The reliable diagnosis of the absence of spermatozoa in a semen sample is a criterion for diagnosing male infertility.[44] Not only absence of sperm count but also the sperm quality is important for fertility. Male infertility is an alarming global health issue that has not been researched or studied to truly understand its magnitude and prevalence. It is an important cause of infertility with a strong impact on the psychology and physiology of couple. It can be due to several reasons. There is still a great need for further research into underlying etiology and treatment of male infertility. Therefore, it's the need of the hour to look into the factors which are causing such a rise in male infertility and attempts should be made to control such factors in near future.

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Conflict of interests

The author(s) declare that they have no conflict of interests.

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