THE STUDY OF SEMEN QUALITY FOR EVALUATION OF
MALE FACTOR INFERTILITY
Mafruha Khanam1* Aditi Banerjee2 Jesmin Jerin3 Mohamed Mazih Fazyl4

Abstract
Background: The global infertility rates are increasing at an alarming pace. Standard semen analysis is the basic and initial investigation in clinical practice for understanding male infertility. The objective of this study is to evaluate male infertility by studying the quality of semen according to the WHO protocols, identify the semen abnormalities to correlate the relationship between semen parameters and infertile couples.

Materials and methods: This is a cross-sectional prospective study conducted in Surgiscope Fertility Centre, Chattogram, Bangladesh over 9 months on 1348 couples who received services from the fertility centre from November 2018 to July 2019. Multiple variables were selected such as, patient parameters like age of the couple, smoking history, duration of infertility, semen abnormalities which were recorded and statistically analyzed. The procedure and reference values were according to the WHO guidelines 2010.

Results: The minimum age of the patients was 19 and the maximum age of the patients was 60 with a mean age of 33.3 +/- 0.5 years. The majority of the patients whose wives conceived were between the ages of 34 to 44 years and the lowest number of patients whose wives conceived were 55+ years. Moreover, 359 patients (26.63%) conceived while 989 patients (73.37%) did not conceive. No significant relationship between the number of conceptions and addiction was recorded. There is a statistically significant negative relationship between total motile sperm and age of patient and a marginally positive relationship between the number of sperm and total motility.

Conclusions: This study showed a higher incidence of abnormal semen quality among husbands of infertile couples in our population. This revealed the importance of emphasizing on the need for managing male infertility.

Key words
Semen analysis; Infertility; Male partners; World Health Organization Criteria 2010.

Introduction
The ascending rates of infertility is of global concern, affecting approximately (8-12) % of couples worldwide1-3. Infertility is characterized as the failure to establish a clinical pregnancy after one year or more despite having regular unprotected coitus2. There are two types, primary and secondary infertility.

Previously, infertility was considered to be a female incompetence, but recent data has shown that a male factor is solely responsible in about 20% of infertile couples and contributory in another 30-40%4. Male infertility, which is rarely acknowledged, contributes to half of all cases. Another research attributed about 30% of infertility is due to female problems, 30% to male problems, and 30% to combined male/female problems while in 10% there is no recognizable cause1,3. All male infertility factors are almost always identified by abnormal semen analysis. It forms the basic investigation protocol for infertile couples as its role in the assessment of male factor infertility is critical, thus, physicians invariable rely on semen analysis for diagnosing male infertility1,4. Prevaling semen analysis is considered a routine assessment in evaluating male fertility and the alternative measure of male fecundity in clinical practice. Routine semen analysis includes the evaluation of the essential parameters like volume, pH, sperm count, motility and sperm morphology. Sperm count, motility and morphology are essentially considered to be imperative parameters of male fertility5. The important risk factors of male infertility might be the less count in rapid, slow and progressive movement of the sperms, reduced sperm count or no count, decrease in volume, infectious semen, erectile dysfunction, stress, weight variation, age, exposure to toxins or chemicals, family history of infertility, weak childhood urological history might cause infertility6,7,8.
During ejaculation, semen is produced from a concentrated suspension of spermatozoa stored in paired epididymis mixed with fluid secretion from the accessory sex glands. The total number of spermatozoa reflect sperm production by the testis and the patency of post testicular duct system. The total fluid volume reflects the secretory activity of glands. The nature of spermatozoa (Concentration, vitality, motility and morphology) and the composition of seminal fluid are important for sperm function.

Semen analysis is the fundamental and minimum investigation for diagnosing male factor infertility. As a matter of fact, it is the first step towards diagnosis of male factor in fertility work up. Semen analysis is performed widely in all accredited pathological laboratories of Bangladesh. Our Surgiscope fertility centre follows the methods and the reference values according to the latest WHO manual for examination of semen (WHO guidelines 2010). The study was conducted in an attempt to assess and evaluate the semen characteristics of all the males in our population, irrespective of primary or secondary infertility. The study also reflected the percentage of infertility in male partners of couples presenting at our fertility centre. The objective of the study is to evaluate the characteristics of abnormalities in the semen of male partners of couples presenting with infertility, and to identify the contribution of male factors to overall infertility problem in our environment.

**Materials and methods**

This is a cross sectional prospective study which was performed on 1348 couples & conducted at the Surgiscope Fertility Centre, Chattogram, Bangladesh over 9 months from November 2018 to July 2019. The consent of the couples was obtained faithfully in the form of informed written format after explaining how their data would be used for this study. Inclusion criteria was the samples were collected from patients with (3-5) days abstinence from ejaculation using masturbation. No prior usage of antibiotics and spilled sample collection were avoided. Cases that did not follow the above-standard criteria were not included in the analysis. Finally, using WHO 2010 standard semen analysis was carried out by determining semen appearance, volume, pH, sperm concentration, motility, morphology, viability, and the presence of WBC or RBC. Exclusion criteria was patients with severe endometriosis, hypospadias, retrograde ejaculation, and bilateral tubal block. Data have been interpreted and evaluated carefully for statistically conclusion.

**Ethical clearance was obtained from the relevant authority.**

**Semen analysis**
The sample was collected by maintaining privacy of the patient and was transported to the laboratory within 1 hour of collection in a container at room temperature (37°C) for liquefaction. The appearance of the semen was determined at half hourly interval. The volume was measured by aspirating the semen into a graduated syringe and the pH was assessed by using pH paper. Microscopic examination of unstained preparation of fresh semen was conducted using a light microscope in a Makler counting chamber. Motility data was assessed as percentages of (A) fast forward progressive (B) slow forward progressive (C) non-progressive and (D) immotile spermatozoa. Morphology data were taken using Tygerberg’s strict criteria. Finally, vitality was analyzed by eosin-nigrosin staining procedure.

The reference used are as follows:

**Normal seminal fluid analysis**

<table>
<thead>
<tr>
<th>Semen parameter</th>
<th>WHO Guideline (2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>&gt; 1.5 ml</td>
</tr>
<tr>
<td>pH</td>
<td>&gt; 7.2</td>
</tr>
<tr>
<td>Sperm concentration (Per ml)</td>
<td>&gt; 15 million spermatozoa per ml</td>
</tr>
<tr>
<td>Total sperm number (Million)</td>
<td>&gt; 39 million spermatozoa per ejaculate</td>
</tr>
<tr>
<td>Total motility (Percentage of progressive motility and non-progressive motility)</td>
<td>&gt; 40% motile or &gt; 32% with progressive motility</td>
</tr>
<tr>
<td>Vitality (% Live)</td>
<td>&gt; 58% live spermatozoa</td>
</tr>
<tr>
<td>Sperm morphology (percentage of normal)</td>
<td>&gt; 4%</td>
</tr>
</tbody>
</table>

**Statistical analysis**
The variables selected were: patient parameters like age of patient, age of wife, abstinence, addiction, duration of marriage, conception, duration of pregnancy attempt, miscarriage. Parameters related to semen analysis include seminal appearance, seminal liq, time, seminal const, semen volume, pH, sperm concentration, total sperm number, total motility, progressive motility, non-progressive motility, normal sperm morphology, abnormal sperm morphology. Of these parameters, conception, miscarriage, seminal appearance was recorded as categorical variables. Other parameters were
taken as continuous variables. An Ordinary Least Squared (OLS) regression model was used in the statistical analysis. A p-value of <0.05 was considered to be significant. Data were entered in SPSS version 17.

**Results**

The age of the patient and his wife were recorded. The minimum age of the patients was 19 and the maximum age of the patients was 60 with a mean age of 33.3 +/- 0.5 years.

Figure 1 shows that the majority of the patients whose wives conceived were between the ages of 34 to 44 years, followed by 29 to 33 years old and the lowest number of patients whose wives conceived were 55+ years.

![Age distribution of successful conception](image)

**Table I:** Regression between number of conceptions and addiction.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>Number of obs</th>
<th>F(1,772)</th>
<th>Prob &gt; F</th>
<th>Adj R-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>.065437462</td>
<td>1</td>
<td>.065437462</td>
<td>774</td>
<td>0.34</td>
<td>0.5576</td>
<td>0.0004</td>
</tr>
<tr>
<td>Residual</td>
<td>146.799756</td>
<td>772</td>
<td>.1901477326</td>
<td></td>
<td></td>
<td>0.0008</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>146.859873</td>
<td>775</td>
<td>.189985994</td>
<td></td>
<td></td>
<td>.4306</td>
<td></td>
</tr>
</tbody>
</table>

Table II shows that among 1348 patients, 359 patients (26.63%) conceived while 989 patients (73.37%) did not conceive.

**Table II:** Overall Conception Rate.

<table>
<thead>
<tr>
<th>Conceived</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>989</td>
<td>73.37</td>
<td>73.37</td>
</tr>
<tr>
<td>YES</td>
<td>359</td>
<td>26.63</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>1,348</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Table III shows the regression between total motile sperm, the age of the patient and number of total sperm (In millions) which indicates that there is a statistically significant negative relationship between total motile sperm and age of patient. There is also a marginally positive relationship between the number of sperm and total motility.

**Table III:** Regression Between Total Motile Sperm (NP+P, %) and Age of Patient and Number of Total Sperm (In millions).

<table>
<thead>
<tr>
<th>Constant</th>
<th>Age</th>
<th>Number of Sperm (In millions)</th>
<th>R Squared</th>
<th>No. of Obs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75.029</td>
<td>-.3668753***</td>
<td>0.0868</td>
<td>1,189</td>
</tr>
</tbody>
</table>

Dependent Variable: Total Motile Sperm.
Discussion

The study attempted to identify the male factor infertility with regard to abnormal semen parameters and to form a model for success for more accurate prediction. The variables studied include factors such as patient parameters like age of patient, age of wife, abstinence, addiction, duration of marriage, conception, duration of pregnancy attempt, miscarriage. Parameters related to semen analysis include seminal appearance, seminal liquid, seminal consistency, total sperm number, total motility, progressive motility, non-progressive motility, normal sperm morphology, abnormal sperm morphology. Our analysis found significant correlation between total motile sperm and age of patient and number of sperm and total motility.

Semen analysis is an extremely common, yet most under interpreted test carried out in evaluation of infertility of a couple. Females have often been the target of society for infertility, however, development of assisted reproductive techniques have proven males to be an equal contributor to this problem. Semen analysis is the cornerstone of the laboratory evaluation of the infertile male and helps us to define the severity of the male factor; it gives indications on testicular function and of the integrity of the male genital tract which may facilitate treatment plans.

Our population size was of 1348 individuals having a mean age of 33.3 +/- 0.5 years with a minimum age of the patients was 19 and the maximum age of the patients was 60. A relatively similar mean age of 34 years in accordance to our finding has been reported by other authors. In our study, majority of the patients whose wives conceived were between the ages of 34 to 44 years, followed by 29 to 33 years old and the lowest number of patients whose wives conceived were 55+ years. This has been stated by another study that male fertility usually peaks at around 35 years of age and declines after 45 years of age.

Our has shown that there is no effect of addiction (Smoking) of the male partner and conception. However, this may possibly be due to the low number of participants (774) respondents compared to the total number of participants. Other authors have reported similar findings that natural conception in couples with a smoking male partner fail to demonstrate a significant reduction in fecundity. In contrast, a study has shown the reduced creatine kinase activity in sperm caused by smoking may potentially impair sperm energy homeostasis and is associated with lower sperm motility. Sperm Creatine Kinase (CK) is an energy reservoir for the rapid buffering and regeneration of adenosine triphosphate and plays an important role in sperm motility. An in vitro study revealed the inhibitory effects of tobacco smoking on capacitation-associated protein phosphorylation in human spermatozoa, leading to changes in posttranslational modifications through which exposure to tobacco can negatively affect sperm functions and cause fertility problems. First and foremost, tobacco smoking leads to reduced semen quality including semen volume, sperm density, motility, viability, and normal morphology in smokers. However, much is known now about the carcinogens in tobacco cigarette smoke and their resultant effect on organs like lungs and urinary bladder, their effects on fertility status have been less documented.

According to our study, among 1348 patients, 359 patients (26.63%) conceived while 989 patients (73.37%) did not conceive. This implies that a vast majority of infertility problems are due to primary infertility. Another study in accordance to our results have shown that the overall rates of primary and secondary infertility were 67.37 and 32.63%. According to this study, the sperm abnormality is the major cause for infertility (45.1%) among primary infertile men compared to secondary infertility (20.3%).

Among all the conventional parameters, sperm motility is the most relevant indicator of fertilization. Assessment of sperm motility is essential as the spermatozoa have to travel in the female genital tract to fertilize the oocyte, a requisite of normal pregnancy. Our study has shown a statistically significant negative relationship between total motile sperm and age of patient. Similar findings have been reported in a demonstrating decreased sperm motility among older men of greater than 50 years compared to younger subjects. This study showed that the percentage progressive motile spermatozoa (a + b) was significantly lower in age of the patient above 50 years as compared with the younger age group of 21-30 years. As stated earlier, sperm motility is an essential requisite and a predictor of fertility, it comes with sperm maturation in their passage through the epididymis. Motility is also a determinant
of how efficiently the sperms penetrate the cervical mucus transport through the female genital tract, and penetration through the corona radiate and zona pellucida before oocyte fertilization. Increasing in age significantly influences semen parameters required for healthy male fertility. Age related changes on the seminal parameters were also evaluated in our study, it was noted that mean sperm counts, total motility and normal morphology revealed a decline in the average values of these parameters with age, and the results were similar to another studies.

Limitation
The sample population was restricted as the study was based on a single centre. We also excluded patients with severe endometriosis, hypospadias, retrograde ejaculation, and bilateral tubal block.

Conclusion
Male factor infertility contributes to significant cases of infertility worldwide. Semen analysis is the first step towards diagnosis of male factor in fertility work up. This study showed a higher incidence of abnormal semen quality among husbands of infertile couples in our population, signifying that we need to acknowledge the contribution of male partners to infertility. This will also help to reduce the stigmatization of women for infertility, as seen in most developing countries.

Recommendation
Further studies should be carried out involving large number of participants in multiple centres to specify the correlation between male infertility and infertile couples by studying the quality of semen according to the WHO protocols.

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Contribution of authors
MK- Conception, design, acquisition of data, drafting & final approval.
AB - Acquisition of data, interpretation of data, data analysis, critical revision & final approval.
JJ- Acquisition of data, interpretation of data, critical revision & final approval.
MMF - Acquisition of data, interpretation of data, data analysis, critical revision & final approval.

Disclosure
All the authors declared no competing interests.

References


