Seminogram in male partners of Infertile Couples
Bodal VK1, Malik R2, Kaur S3, Bal MS4, Kaur P5, Bhagat R6, Singh KD7, Goel A8

ABSTRACT

Background: Infertility is defined as failure to conceive within one or more years of regular unprotected coitus. The infertility state is dependent on the female factor as well as masculine factor; an altered masculine factor is designated when any cause or causes of infertility reside in the male. The masculine factor as a cause of infertility is present in 40-50% of cases hence the importance of an integral evaluation of the male alterations and its fertility.

Objectives: The present study aims to assess the seminal patterns of male partners of 100 infertile couples for various parameters and their possible contribution to infertility.

Material and Methods: The present study was conducted on male partners of 100 infertile couples who were referred by Gynecology and Obstetric department to Pathology Department, Government Medical College, Patiala for semen examination. The semen was collected by masturbation in all cases in a clean dry detergent free container. After liquefaction and mixing, basic analysis was done which includes volume, viscosity, pH, spermatozoal concentration, motility and morphology. Data was evaluated by means of chi-square test.

Results: Of 100 seminogram, 43% showed alterations in the seminal indexes; with asthenospermia in 39.5%, Oligoasthenospermia in 30.2%, Oligospermia in 16.2%, and Azoospermia in 13.9%.

Conclusion: Male factors were mostly responsible as a cause of infertility. Asthenospermia was the most common type of semen defect present in these infertile males. Most of the males with semen defect were of age group >30yrs. Incidence of semen defect among males increased with duration of infertility.

Keywords: Infertile couple, seminogram, sperm count, sperm motility

Introduction
Childlessness may be a tragedy to the married couple and can be a cause of marital upset as well as of personal unhappiness. The having of children cements a marriage. The desire of women for children is usually stronger than self-interest in beauty and figure, and may be stronger than the claims of a career. [1]

Infertility is defined as a failure to conceive within one or more years of regular unprotected coitus. Primary infertility denotes those patients who have never conceived. Secondary infertility indicates previous pregnancy but failure to conceive subsequently. [2] The incidence of infertility in any community varies between 5 and 15 percent. [3] The male is directly responsible in about 30-40 per cent, the female is about 40-55 per cent and both are responsible in about 10 per cent cases. The remaining 10 percent is unexplained in spite of thorough investigations. [2]

Male infertility can be due to Pretesticular, Testicular, Post testicular cause.

Pre-testicular causes of infertility:
Gonadotrophin deficiency, Thyroid dysfunction, Hyperprolactinaemia, Antihypertensives, Antipsychotics, Genetic disorders, Erectile dysfunction, Impotence.
Testicular causes of infertility: Immotile cilia (Kartagener) syndrome, Cryptorchidism, Infection (mumps, orchitis), Toxins, Varicocele, Immunologic, Sertoli cell only syndrome, Oligoasthenoteratozoospermia.

Post-testicular causes of infertility
- Congenital like absence of vas deferens (cystic fibrosis), young's syndrome.
- Acquired causes are infections like tuberculosis, gonorrhoea etc. [2]

Impaired sperm mobility, presumably because of faulty maturation or storage of spermatozoa in the epididymis, is also included among the post testicular causes of infertility. [4] For adequate spermatogenesis, the testicles must lie in its correct position in the scrotum where the temperature is slightly cooler than elsewhere in the body. The factors which raise the scrotal temperature can adversely influence spermatogenesis, e.g. the occupation of men who work as strokers or in blast-furnaces are subjected to excessive heat. [5]

Several other factors are also known to effect fertility. These include age, duration of sexual exposure, frequency of coitus, nutritional, environmental and social factors. Deficiency of certain essential nutrients as proteins, vitamins may cause reproductive failure. Environmental pollutants, chemicals and excessive heat have also been linked with impairment of fertility particularly in the male. Lifestyles e.g. smoking, excessive alcohol consumption, drug abuse and even over exercise have been linked both in female and male reproductive failure. Cross cousin marriage plays an important role in infertility. Male factors contribute to almost 50% of infertility in infertile couples while the infertility in the remainders may be either due to a female factor or a combination of male and female factors. In approximately 10-15% of the infertile couples no apparent cause can be found and such cases are categorized under unexplained infertility. The involvement of emotional factors in infertility is an accepted fact. [5]

WHO Guidelines 2010 for Normal seminal fluid analysis
- Volume - > 1.5 ml
- pH - 7.2 to 8.0
- Liquefaction time - 20 to 30 min
- Sperm concentration - >15 million/ml
- Total motility - 40%
  (Progressive motility + non progressive motility)
- Progressive motility - 32%
- Morphology - > 4% normal forms [6]

Abnormal Seminal fluid analysis
- Oligozoospermia – reduced sperm numbers
- Asthenozoospermia - reduced sperm motility
- Teratozoospermia - increased abnormal forms of sperm
- Oligoasthenoteratozoospermia - all sperm variables are subnormal.
- Azoospermia - no sperm in semen
- Necrospermia – all the spermatozoa present are dead. [7]

Since the beginning of time human infertility has been a source of personal misery, and even of national crises. It was once, and still is in some communities, regarded as a disgrace, as a mark of Divine displeasure, as grounds for divorce and even for compulsory suicide (on the part of the woman only). The Egyptians, Greeks and earlier civilizations all had empirical treatments – love potions, amulets, prayers, sacrifices and the like. Although the female partner was generally blamed, the Greeks at least were aware of male infertility. [1]
The present study was undertaken to evaluate the male factor in infertility.

**Material and methods**
The present study was conducted on male partners of 100 infertile couples who were referred to the department of pathology at GMC Patiala for semen examination. The semen was collected by masturbation in all cases in a clean dry detergent free container. After liquefaction and mixing, basic analysis was done which includes volume, viscosity, pH, spermatozoal concentration, motility and morphology. The present study aims to assess the seminal patterns of male partners of 100 infertile couples for which semen sample were collected and subjected to physical examination as well as microscopy for various parameters and their possible contribution to infertility. The patients enrolled in the study were explained about the procedure after obtaining written informed consent. After taking relevant history, biochemical investigations were also done.

**Inclusion criteria:**
Infertile couples who were living together for more than one year and had regular unprotected sexual intercourse.

**Exclusion criteria:**
Infertile couples who were using some method of contraception.

Patients having erectile dysfunction due to:
- Drugs such as SSRIs, beta blockers.
- Psychological causes like performance anxiety, stress, clinical depression, schizophrenia etc.

**Duration of Abstinence**
Patients were asked to come after a period of abstinence of 2 to 4 days.

**Collection and Transportation**
A clean dry detergent free glass container with a wide opening was used for the collection of ejaculate. The semen was collected by masturbation in all cases in premises of our clinical laboratory. In cases of local patients who preferred to collect at home, they were advised to bring it within half an hour to the laboratory without exposing it to the extremes of temperature. After liquefaction and mixing, basic analysis was done which includes volume, viscosity, pH, spermatozoal concentration, motility and morphology.

**Volume:** The volume of the sample was measured in a graduated tube or a small cylinder.

**pH:** The pH of the semen was measured with pH paper.

**Viscosity:** The viscosity was measured by gently aspirating it into a wide bore pipette allowing the semen to drop by gravity and observing the length of any thread.

**Motility of Sperms:** Sperm Motility was estimated by mounting a drop of liquefied semen on a slide and covering it with cover slip.

**Sperm count:** Sperm count was done in Neubauer chamber. After liquefaction has taken place, the specimen was gently mixed. The semen was drawn to the 0.5 mark of a W.B.C. pipette and the special semen diluting fluid was drawn to the 11 mark and both were mixed well. Alternatively, bulk method was used in which, in a small test tube, 0.2ml semen was taken with auto-pipette and 0.38ml of diluting fluid was added to it and both were mixed well.

The composition of the diluting fluid was as follows:
- Sodium bicarbonate : 5gm
- Phenol or formalin (Neutral) : 1ml
- Distilled water : 100ml

The Neubauer Chamber was charged and after allowing the sperms to
settle they were counted in the 4 corner squares.

Sperm concentration were calculated by the following formula

\[ \text{Sperms/cc} = \frac{N \times 20 \times 1000}{4 \times 0.1} \]

N – Number of sperms in 4 large squares.
Dilution is 1 in 20
Depth of the chamber – 0.1 mm

**Morphology of sperms** After liquefaction, little amount of semen was taken and thin smears prepared. PAP staining was done to study morphology of sperms.

**Result of PAP staining:**
- Spermatozoa: Blue
- Acrosomes: Pink
- Postacrosomes: Dark blue
- Tail: Pink

The findings were recorded and analyzed as per WHO guidelines for semen analysis. The significance of relations among the variables was evaluated by means of chi-square test.

**Results**

Of the total 100 infertile couples attending the infertility clinic the male factor was responsible in 43% cases as a cause of infertility and 57% males were normospermic (Table 1). Rest 43% had some type of semen defect in which asthenospermia constitutes maximum of (17%), followed by oligo-asthenozoospermia (13%), oligozoospermia (7%) and azoospermia (6%). (Table 2)

<table>
<thead>
<tr>
<th>Table 1: Incidence of abnormal seminogram among infertile couples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Males with normal seminogram</td>
</tr>
<tr>
<td>Males with abnormal seminogram</td>
</tr>
<tr>
<td>Total No. of Infertile Couples</td>
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<thead>
<tr>
<th>Table 2: Distribution of male partners of infertile couples on basis of semen defect</th>
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</thead>
<tbody>
<tr>
<td><strong>Semen Defect Present</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Normospermia</td>
</tr>
<tr>
<td>Asthenospermia</td>
</tr>
<tr>
<td>Azoospermia</td>
</tr>
<tr>
<td>Oligoasthenozoospermia</td>
</tr>
<tr>
<td>Oligozoospermia</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
Couples with duration of marriage >3-6 yrs were 45% (maximum), followed by couples with duration of marriage 1-3 yrs (44%), >6-9 (10%), and least no. of those whose duration of marriage >9-12 yrs (1%). The incidence of semen defect was highest among couples with duration of marriage >3-6 yrs (51.1%), followed by those with duration of marriage 1-3 yrs (37.2%). The incidence of semen defect as a cause of infertility when compared with duration of marriage was found to be statistically significant (\( p = 0.022 \) \( \chi^2 = 23.773 \) (Table 3).

**Table 3: Incidence and comparison of type of semen defect among male partners of infertile couples on basis of duration of marriage**

<table>
<thead>
<tr>
<th>Duration of marriage (Yrs)</th>
<th>Final Diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>OS</td>
</tr>
<tr>
<td>1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Of Male Partners</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>% within Final Diagnosis</td>
<td>49.1</td>
<td>42.9</td>
</tr>
<tr>
<td>&gt;3-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Of Male Partners</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>% within Final Diagnosis</td>
<td>40.4</td>
<td>42.9</td>
</tr>
<tr>
<td>&gt;6-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Of Male Partners</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>% within Final Diagnosis</td>
<td>10.5</td>
<td>0</td>
</tr>
<tr>
<td>&gt;9-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Of Male Partners</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>% within Final Diagnosis</td>
<td>0</td>
<td>14.3</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>7</td>
</tr>
</tbody>
</table>

NS: Normospermia, OAS: Oligoasthenozoospermia, OS: Oligoazoospermia, AS: Asthenospermia, AZS: Azoospermia

Majority of male partners with semen defect i.e. 24 (55.8%) was of age group >30 yrs, followed by 17 (39.5%) in age group >25-30 with only 2 (4.6%) males with abnormal semen in age group ≤ 25. And asthenospermia is the most common type of semen defect in age groups of >25-30 yrs (12.1%) and in >30 yrs (31.3%), followed by oligozoospermia (6.9%) in age group >25-30 and oligo-astheno-zoospermia (28.1%) in age group >30 yrs. The incidence of semen defect was statistically significant (\( \chi^2 = 24.665, \ p=0.002 \) when compared among different age groups. Maximum number of cases of asthenospermia and oligoasthenospermia were in the age group of >30 years. (Table 4)
Table 4: Incidence and comparison of type of semen defect among male partners of infertile couples in different age groups

<table>
<thead>
<tr>
<th>Age Group (Yrs)</th>
<th>Final Diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>OS</td>
</tr>
<tr>
<td>≤ 25</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>% within Final Diagnosis</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>&gt;25-30</td>
<td>41</td>
<td>4</td>
</tr>
<tr>
<td>% within Final Diagnosis</td>
<td>70.7</td>
<td>6.9</td>
</tr>
<tr>
<td>&gt;30</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>% within Final Diagnosis</td>
<td>25</td>
<td>9.4</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>7</td>
</tr>
</tbody>
</table>

NS: Normospermia, OAS: Oligoasthenozoospermia, OS: Oligoazoospermia, AS: Asthenospermia, AZS: Azoospermia

Discussion

In the present study it was found that the male factor was responsible in 43% cases as a cause of infertility as shown in Table 1. Roland (1968) conducted a study and found that contribution of male factor to infertility was approximately 40%. Raymont et al (1970) collected data of 500 infertile couples and found that male factor was responsible in 31.5% cases. Behrman et al (1975) observed that the male factor as a cause of infertility was present in about 30-35% cases. Dor et al (1977) investigated 665 infertile couples, male infertility factor was found to be present in 28%. Marshall (1978) conducted study in USA in which it was found that male was responsible in about 30% cases as a cause of infertility and 30% had combined factor both male and female factor.

Dawn (1980) conducted study and found that the incidence of infertility was 10% according to hospital statistics and 2.5% in general population. Male factor was responsible in 25% cases and 20% cases have combined male and female factors. Rajan et al (1981) investigated 1268 infertile couples over a period of 4 years 6 months, male factor was found responsible in 54% couples. Andrews et al (1986) analyzed national survey of family growth in USA and found that male factor in infertility accounted for approximately 40% of the cases. Bhide (1990) investigated 768 infertile couples over 8 year period and found that in 213 (28%) couples’s infertility was attributable to male factor. Rajan (1990) evaluated 6009 infertile couples over 17 years and it was found that male and female factors causing infertility occurred with almost same frequency i.e. 40.7%, 38.13%, respectively.

The results of male factor as a cause of infertility in the present study was similar to studies done by Roland (1968), Andrews et al (1986), Rajan (1990) and Bayasgalan (2004). However, contribution of male...
factor as a cause of infertility varies in studies done by Raymont et al (1970), Behrman et al (1975), Don et al (1977), Marshall (1978) and Dawn (1999) from the present study. The possible reason for this discrepancy might be because of variation in geographical factors and small sample size in present study. In the study done by Bhide et al (1990) the male factor as a cause of infertility was approximately 20-25% while in present study it was 43%, the possible reason for this discrepancy might be the study sample which in present study was small i.e. 100 compared to 778 couples in Bhide et al study.

In the present study, incidence of type of semen defect was studied among male partners of infertile couples and it was found that asthenozoospermia was present in 17 % of male partners of infertile couples as shown in Table 2. In a study from university college hospital Ibadan done by Adenijiv et al (2003) it was found that asthenozoospermia was the most common seminal index found altered among males of infertile couples i.e. 27.8% followed by oligo-asthenozoospermia (25.5%), azoospermia in 6.7% cases. [18]

Ugboaja et al (2010) studied the pattern of seminal fluid abnormalities in male partners of infertile couples in south-eastern Nigeria over a period of 12 months and it was found that out of the 348 semen sample reports evaluated 237 had semen fluid abnormalities. Asthenozoospermia (16.7%) was the single main abnormality followed by oligoasthenozoospermia (14.7%). [19]

Salgado et al (2003) conducted a study on 571 infertile couples and observed that asthenozoospermia was present in 8.89% cases. [20] Percentage of asthenozoospermia cases as a cause of infertility in present study is comparable with the study conducted by Ugboaja et al (2010) but the percentage of asthenozoospermia cases were different in studies conducted by Adenijiv et al (2003) and Salgado et al (2003). The reason for this difference might be that the studies were conducted in different geographical areas where different environmental factors affect seminal index.

In present study majority of male partners of infertile couples with semen defect i.e. 24 (55.8%) was of age group >30 years followed by 17 (39.5%) in age group around >25-30 years with only 2 (4.6%) males with abnormal semen indexes in age group ≤25 years. The incidence of semen defect was statistically significant when compared among different age groups as shown in Table 4. Warner (1963) in his 25 years study of sterility treatment of 1553 couples found that mean age of males among infertile couples was 33.1 years [21]. Cates et al (1985) investigated 5800 sterile couples and observed that majority of the couples were between 25-34 years of age in Asia. [22]

Marimuthu et al (2003) conducted semen analysis of subjects attending the fertility clinic for last 11 years, observed that the average age of men attending the infertility clinic was 31.2 years. [23] In another study conducted by Salgado et al (2003) in which he studied the seminogram of 571 couples that consulted for infertility, they observed that the majority of men with altered seminal indexes were of age group 31-39 years. So as per above most studies showed that majority men with abnormal seminal indexes belong to age group >30 years as was in present study. The reason for alteration of seminal indexes in this age group was that with increase age of male there occurred physiological alteration in various seminal parameters.
More over majority of our population is still uneducated they always report late to infertility clinic and in our society males are rarely considered at problem, so they rarely get themselves tested for sterility and seminal examination.

In present study the incidence of semen defect was highest among couples with duration of infertility >3 - 6 years (51.1%) followed by those with duration of marriage 1 - 3 years (37.2%) as shown in table 3. Warner (1963) analysed that in 33% of his patients the infertility span was 3-5 years. Insler et al (1981) reported that mean duration of infertility in his study was 34.5 months. [24] The results of our study were approximately comparable with the previous studies coated above. The reasons for increased incidence of semen defect with increasing duration of infertility might be because of changing characteristics of components of semen with increasing age of men, more over it is quiet prevalent in our society that male partners of infertile couples themselves get investigated in last when it is proved by every means that female is not at problem. The importance of duration of infertility/marriage is that longer is the infertility span, more intense is the treatment required and more elusive are the pregnancy rates.

It was concluded from the present study that the male factor was responsible in 43% cases as a cause of infertility and asthenospermia (17%) was the most common type of semen defect present in these infertile males followed by oligo-asthenozoospermia (13%). Majority of male partners with semen defect i.e. 24 (55.8%) were of age group >30yrs and were from urban background. Uneducated males had high incidence of semen defect (65.7%). Also, with increase in the duration of infertility, the incidence of semen defect among males increased. It was highest among couples with duration of infertility >3-6 years i.e. 51.1%, followed by those with duration of infertility 1-3 years i.e. 37.2%.

References

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