Comparative Study of Sperm Concentration in Pakistani Men

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Abstract
Background: To determine the sperm concentration of proven fertile males and to compare this with that of infertile males.

Methods: In this comparative study semen samples of fifty husbands of pregnant women attending the antenatal clinic were obtained. Another fifty infertile men were inducted into the study as a control group. Inclusion criteria for the proven fertile males was the pregnancy achieved within one year of marriage with successful coituses. For the infertile males the inclusion criteria was failure to achieve pregnancy without the use of assisted reproductive techniques, with no infertility factors in the female partner. The exclusion criteria was secondary infertility, tuberculosis, mumps, orchitis, any chronic debilitating illness, varicocele, sexually transmitted diseases, any drug affecting male fertility e.g. beta-blockers, anti-neoplastic agents etc. The semen samples were obtained after 3 to 4 days of sexual restraint at the laboratory. A drop of 10 – 15 μl of semen in the center of Makler’s chamber was placed and covered with cover glass. Total number and motile number of sperms in 10 squares of the grid under phase contrast microscope at x20 magnification were counted. Three observations were taken and an average number of total sperm count and motile sperm count were calculated. This gives number of sperms x 10⁶/ml. Chi- square test was used. P-value <0.05 was considered statistically significant.

Results: The total sperm count ranged from 20 to 179 millions/ml in the proven fertile male group and from 0 to 169 millions/ml in the infertile group. Total sperm count was significantly higher in proven fertile males as compared to the infertile males group (p < 0.004)

Conclusion: Sperm concentration is useful in in-vivo situation to find males having a greater chance of infertility problem.

Key Words: Sperm concentration, Fertile males, Semen parameters.

Introduction
The current definition of infertility is defined by the American Society for Reproductive Medicine as the inability to achieve a pregnancy through natural means after 1 year.1 The definition of male infertility however is less clear. Diagnosis is usually based purely on abnormal semen analysis. Unfortunately, most aspects of sperm distribution are still indistinct in both normal and abnormal semen although there are plenty of studies available. It has been found that the males contribute about 30 to 40 % to infertility.2 Male partners in couples have been tried to be identified as having significantly lower chance of fertilization in vitro or in intruterine insemination (IUI) programmes by the doctors in the last twenty years or so.3-5 The techniques such as In-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) performed for male factor have shown to have significantly higher chances of conception.6 The basis of the evaluation of male reproductive health is the estimation of semen parameters like sperm concentration, motility and morphology.7 The first step in the investigation of men suspected of having male factor infertility who are husbands of women attending infertility clinics is a semen analysis.8 The expectation of conception and introduction of therapy for achieving pregnancy are both dependent on the result of this investigation.

Decreased sperm concentration has been associated with decreased fertility. Although, fertile population have rarely been studied, widely used thresholds for normal semen measurements have been published by the World Health Organization; however, the available norms for sperm concentration, motility, and morphology fail to meet the rigorous clinical, technical, and statistical standards. 9 In view of these constraints, the nomenclature in the WHO manual for semen evaluation was changed from ‘normal’ to “reference” values. 7 It was concluded in a study that thresholds of less than 5% normal sperm morphology and progressive motility of less than 14% should be used to identify the infertile males.10 As far as
Subjects and Methods
This comparative study took place at the Railway teaching hospital, Rawalpindi and Islamabad clinic serving infertile couples, from June 2005 to July 2006. Fifty husbands of pregnant women attending the antenatal clinic at Railway hospital, Rawalpindi were employed in the study. Another fifty infertile men were inducted into the study as a control group, who consulted at the Islamabad clinic serving infertile couples, for their issue. Inclusion criteria for the proven fertile males was the pregnancy achieved within one year of marriage with successful coituses. For the infertile males the inclusion criteria was failure to achieve pregnancy without the use of assisted reproductive techniques, with no infertility factors in the female partner. The exclusion criteria was secondary infertility, tuberculosis, mumps, orchitis, any chronic debilitating illness, varicocele, sexually transmitted diseases, any drug affecting male fertility e.g. beta-blockers, anti-neoplastic agents etc. The semen samples were obtained after 3 to 4 days of sexual restraint. The semen sample was allowed to liquefy and then mixed with plastic transfer pipette. A drop of 10 – 15 μl of semen in the center of Makler’s chamber was placed and covered with cover glass. Bubbles were avoided. Once cover glass was placed further lifting or touching was avoided which could disturb the uniform layer of sperms. Total number and motile number of sperms in 10 squares of the grid under phase contrast microscope at x20 magnification were counted. Three observations were taken and an average number of total sperm count and motile sperm count were calculated. This gives number of sperms x 10^6/ml.

Results
The total sperm count ranges from 20 to 179 millions/ml in the proven fertile male group and from 0 to 169 millions/ml in the infertile group. Total sperm count was significantly higher in proven fertile males as compared to the infertile males group (p < 0.004) (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Sperm Count (in millions per ml)</th>
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<tbody>
<tr>
<td>Proven Fertile (n=50) (Mean ± SD)</td>
<td>84.62 ± 17.06</td>
</tr>
<tr>
<td>Infertile (n=50) (Mean ± SD)</td>
<td>58.10 ± 21.90</td>
</tr>
<tr>
<td>p-Value</td>
<td>&lt; 0.004*</td>
</tr>
</tbody>
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* p = Significant

Discussion
To assess the fertility potential of the males it is a routine practice to use semen analysis. However, the role of traditional semen analysis and semen parameters as a prognostic factor of a male’s fertility potential is a continuous debate. Particularly in in-vivo situation, sufficient information is not available on normal and minimal values of sperm morphology, sperm concentration and motility for the establishment of a male’s fertility potential. The primary reason is that the fertile population has rarely been studied. With assisted reproductive technology (ART) becoming a more viable option for couples, more men require a sophisticated interpretation of their semen analysis. In case any of the parameters of the analysis appears “abnormal,” these men are mostly sent for further evaluation to a an infertility clinic. As such, this relies on creation of reference threshold values for semen analysis, which the World Health Organization (WHO) has been attempting to find for the last 30 years. No exact “fertile” or “normal” cutoffs for semen parameters are there. Therefore the only terminology that can be used is a value as “above” or “below” minimum reference values. Since 1987 the WHO has published five editions of the “WHO Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction.” The most recent publication of the 2010 has included lower reference values than the 1999 manual, i.e. a semen volume of 1.5 ml, sperm concentration of 15 million/mL, sperm total motility of 40%, and sperm with a normal morphology of 4% (Kruger criteria).

The estimation of sperm concentration is also one of the basis of the assessment of male reproductive health alongside motility and morphology, when estimating difficulties in achieving a pregnancy and in reproductive toxicology or epidemiology. Though a great and overlapping distribution in the fertile and infertile population has previously been reported for sperm concentration, Bonde et al found sperm concentration and sperm morphology to be most solidly associated with pregnancy when he investigated associations between semen quality measures and pregnancy within six months. Ombel et al, Gunalp et aland Guzick et al calculated cutoff values for sperm concentration i.e. lower threshold values ranging from 9 x 10^6/ml to 14.3 x 10^6/ml. However a threshold value for sperm concentration was not calculated by Menkveld et al, since values of 20 x 10^6/ml were taken as inclusion criteria in their study and they suggested that a threshold value of 20 x 10^6/ml should be used with...
confidence, as it did not have an effect on the results from their fertile population.11
In a study by Slama et al in a population of European couples who had recently conceived a pregnancy, the sperm concentration up to 55 x 10^6/ml was related to time to pregnancy.17 More recently in the study by Keel et al mean sperm concentration of 129.4 x 10^6/ml was found in normal men.20 In another study by Marimuthu et al in New Delhi, India the average sperm count in men attending infertility clinic was found to be 60.6 x 10^6/ml.21 Such variations from one study to another in sperm concentration is probably due to different study designs, population variation; inter group variation, sampling error or sample size etc.
In our study mean sperm concentration was found to be 84 x 10^6/ml in proven fertile males. The results closest to ours were found in a study by Pal et al from India where mean sperm concentration of 68 x 10^6/ml was found in men of proven fertility.22 This similarity could be because of the same geographical region and race.

Semen analysis is the basic laboratory investigation and its results are used to decide the appropriate treatment. It is important that the laboratory and the staff is properly skilled in the performance of semen analyses to ensure an accurate result. It is also important that the laboratories must participate in internal and external quality assurance activities, impart regular training to technical staff and use reliable procedures for making sure that the results are reliable and correct.

Conclusion
1. Sperm concentration is useful in in-vivo situation to find males having a greater chance of infertility problem.
2. Semen analysis, being an important and informative test, requires more meticulous evaluation and quality control at the laboratory side. At clinical side a more objective interpretation is required.

References
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