INTRODUCTION

Infertility is defined as failure of conception after at least one year of unprotected intercourse. Infertility affects approximately 15 - 20% of the population in developed and developing countries. Male factor accounts for subfertility in 20-25% and contributes to a further 30% of well characterized cases.

Male infertility factor is usually defined by abnormal results on semen analysis. Estimates suggest about 6% of men between the ages of 15 and 50 are infertile. Macleod and Gold first reported the differences in sperm characteristics between fertile and infertile men. The sperm morphology is a predicator of man's fertilizing potential. Up to 70-86% of the cells can have morphologically abnormal sperms in normal fertile individuals. An increased number of sperms with tapered heads was found in association with a varicocele in the spermatic cord. Jarow et al. reclassified sub-fertile men into subgroups according to sperm characteristics and found teratozoospermia in 18%, asthenoteratozoospermia in 3.5% and oligo-asthenoteratozoospermia in 23.8%. Sperm motility has been established as a good indicator of semen quality and male fertility. Most males with poor sperm motility also have decreased sperm count or a greater number of abnormally shaped sperms in their semen. Sperm dysfunction may also be due to abnormalities of flagellar movement, failure in sperm zona recognition and failure in the ability to exhibit sperm-oocyte fusion. Pregnancies have been achieved with total sperm count of 1-5 x 10⁶. Crosignani and Walters found that both IVF and IUI were suitable for treating couples suffering from primary infertility due to oligozoospermia, asthenozoospermia or teratozoospermia. They substantiated the inability of IUI to assist in achieving pregnancies for males with asthenozoospermia. Ovarian stimulation with timed intercourse or IUI has been empirically applied alone or in combination for treatment of unexplained infertility, male factor infertility, anovulatory infertility as well as for other cases of infertility. This treatment modality is used when the

ABSTRACT

Objective: To assess the effect of sperm parameters on pregnancy rate following Intrauterine Insemination (IUI) in primary and secondary infertile patients.

Study Design: An observational study.

Place and Duration of Study: The Assisted Conception Unit, Department of Obstetrics and Gynaecology, Services Institute of Medical Sciences, Lahore, Pakistan, from January 2004 to January 2006.

Patients and Methods: One hundred and thirty-six couples, comprising 110 couples with primary and 26 with secondary infertility, of at least one year of infertility, were studied. All underwent a total of 231 intrauterine insemination cycles. A detailed history for infertility evaluation was taken. Hormonal profiles were done on day two of the menstrual cycle. Ovarian stimulation of all patients with Clomiphene citrate was done from day two of the menstrual cycle for 5 days and subsequent transvaginal scans on day twelve, for follicular tracking were done. Semen analysis was done, after 2-3 days abstinence and sperms for intrauterine insemination were prepared according to Density Gradient Sperm Wash Method. IUI was done 36 hours after tracking of the dominant follicles and injection hCG was given for follicular maturation and rupture. The main outcome measures were determining the effect of normal and abnormal sperm parameters on pregnancy outcome, in ovulation induced infertile couples, undergoing intrauterine insemination cycles.

Results: Of the 136 couples, who underwent IUI cycles, 18 had male factor infertility and the rest were with normal semen parameters. An IUI pregnancy rate of 13.7% was seen with a total of 4 conceptions.

Conclusion: This study confirms that IUI, because of its cost effectiveness and minimum complications, can be applied as a first line treatment in infertile couples after considering the sperm parameters.

Key words: Intrauterine insemination. Male factor infertility. Sperm parameters.
female partner has at least one open tube in addition to some ovarian function and the male partner has motile sperm. After ovarian stimulation with CC, IUI is equally effective 24 hours after a spontaneous LH surge or 36 hours after administration of hCG. IUI combined with CC-hCG can be offered as a heavy, safe and non-expensive first line treatment, at least with an inseminating motile count of >1 x 10^6 spermatozoa.

The objective of the present study was to compare IUI pregnancy rate in CC/hCG induced couples with male partners having normal and abnormal sperm parameters.

**PATIENTS AND METHODS**

This descriptive study was carried out at the Assisted Conception Unit, Services Institute of Medical Sciences, Lahore, Pakistan, from January 2004 to 2006. Couples with at least one year of infertility, underwent intrauterine insemination cycles. All couples underwent a basic infertility evaluation. A complete semen analysis, after 2-3 days of abstinence, was done.

Ovarian stimulation of all the 136 females in the study group was done with Clomiphene citrate, 50-200 mg starting from day 3 for 5 days, of the menstrual cycle. Doses were increased from 50 to 100, 150 and 200 mg in subsequent cycles in case of absent ovarian response. Transvaginal scans were done to monitor the ovarian and endometrial responses on day 12 of the menstrual cycle. Presence of at least 2 dominant follicles with a diameter equal to or greater than 18 mm and an endometrial thickness equal to or greater than 8-10 mm were criteria for administering injection hCG, 5000 IU intramuscularly, for follicular release. Intrauterine insemination was done 36 hours after hCG administration.

The WHO criteria was considered for normal semen analysis. A creamy, grayish white or pale white colour of the semen was considered normal while a yellowish colour indicated the presence of increased pus cells in the ejaculate or that the male might have been jaundiced or might be taking some vitamins at the time the semen analysis was performed. Ejaculated semen was viscous but became thin and watery on complete liquefaction after a minimum postejaculatory interval of 30 minutes at room temperature. Semen volume was noted using sterile disposable Pasteur pipette (3 ml).

The samples were mixed with plastic Pasteur pipette. A drop of 5-10 µl of semen was placed in the centre of Horwel chamber and covered with a cover slip, avoiding any bubble formation in between. Total number and motile sperm number were calculated in 10 squares of the grid under phase contrast microscope at x 200 magnification and presented in million sperms/ml or sperms x 10^6/ml. At least three observations were taken and an average number of total sperm count and motile sperm count was calculated.

The following formula was applied to calculate the motility of the sperms.

\[
\%\text{ age of motility} = \frac{\text{Average number of motile sperms}}{\text{Average number of total sperms}} \times 100
\]

A small drop 5-10 µl of liquefied semen sample was placed on a labelled glass slide (Sail Brand 25.4 x 76.2 mm) and covered with a 22 x 22 mm cover slip and observed under a phase contrast microscope at x 400 magnification. The freshly made wet preparation was left to stabilize for approximately one minute. The sperm motility and velocity was assessed at 37°C on a warm stage. Pus cells were estimated in the seminal pleasure at x 400 magnification (high power field of the microscope).

Motility grading was done according to WHO (1999) guidelines. Progression scoring was taken as an average of at least three high power fields, away from the edges, with uniform film so that all the sperms were focused under the same plane.

Sperm morphology was assessed using ocular micrometer (eye 10 x and objective 100 x) at x 1000 magnification of microscope according to WHO criteria (1999). At least two observations were taken especially with sample containing very low normal morphology. In hundred sperms, all head, mid piece and tail defects were noted and mentioned as the percentage of the normal spermatozoa.

Patients were requested to abstain from intercourse 2 days before the day of semen collection. Semen samples were produced by masturbation or intercourse and collected in sterile containers. Each sample was analysed after complete liquefaction at room temperature following the WHO guidelines (1999).

The sperms for IUI were prepared according to the Density Gradient Sperm Wash Method. Cook's medium 40/80 was centrifuged and the supernatant was disposed off. The remaining media containing the sperms was washed with sperm buffer (Cook's buffer), centrifuged again and supernatant discarded. The remaining medium containing sperms was rewashed again with sperm buffer, supernatant discarded and the lower pellet collected and used for IUI.

The prepared sample was checked under the microscope for 100% motility and a minimum of 3-5 million sperms were used for IUI. Using the IUI catheter (Cook's – Australia, Rockett International Pharma, UK), 0.2 ml of prepared semen was injected into uterus after standard preparation. The women remained supine for 20 minutes after the procedure. A pregnancy test was performed 2 weeks after IUI, if menses were not resumed.
All parameters studied in the couples, who underwent IUI, were presented as the mean ± standard error with minimum and maximum ranges, as for example ages of males and females, duration of marriage and infertility and the follicular diameters. Statistical significance was set at p<0.05 and p ≤ 0.01, using the chi-square test and t-test.

RESULTS

Table I shows the intrauterine insemination pregnancy rate of normal males. Mean normal sperm counts (million/ml) showed no statistical difference in the male partners of primary and secondary infertile subjects in all age groups. Mean percentage, excellent motility in male partners of <40 years old, primary infertile females (30.0 ± 1.5) was significantly low compared to that of secondary infertile females (44.0 ± 3.11; t(101) = 3.21; p=<0.01) of the same age group. There was no appreciable difference in good sperm motility in male partners of primary and secondary infertile females. In the male partners of aging primary infertile females (≥ 40 years), sperms with normal morphology were significantly low compared to the male partners of younger (<40 years) primary infertile subjects (t(92)=4.06; p=<0.001). There was no statistical difference in the number of females conceived in primary and secondary infertility cases with normal sperm counts of their male partners (χ²(1)=1.436; p=0.23), regardless of age. However, a strong association between the number of IUI cycles given and the pregnancy rate (χ²(1)=4.524; p=0.0265) was seen.

Table II shows the intrauterine insemination pregnancy rate of male partners with abnormal sperm parameters. Oligozoospermic males had a significantly higher good grade motility (24.54 ± 1.57%) compared to asthenozoospermic males (t (13)= 2.287; p<0.05). There was no significant difference in sperm counts and normal morphologies of sperms in oligozoospermic, oligoasthenozoospermic and asthenozoospermic males. There was no pregnancy in the females with IUI cycles carried by sperms of oligoasthenozoospermic and asthenozoospermic males. Of the 110 couples, females of male partners diagnosed with male factor infertility underwent 29 IUI cycles and showed a conception rate of 22.2% with an IUI pregnancy rate of 13.7%.

DISCUSSION

This study was carried out to determine the intrauterine insemination pregnancy rate in females of male partners with normal and abnormal sperm parameters.

A number of retrospective studies have shown that sperm parameters are, on average, superior in fertile males compared to the infertile. In the present study, fertile male partners had superior sperm parameters compared to male partners with male factor infertility. No difference was observed in the mean normal sperm counts of the male partners of primary and secondary infertile females in all age groups of infertile female subjects. Sperm motility has been established as a good indicator of semen quality and male fertility.

The present study showed mean percentage excellent motility in male partners of <40 years old primary infertile females to be significantly low (p<0.01) compared to that of male partners of secondary infertile females of the same age group. There was no difference in good sperm motility in male partners of primary and secondary infertile females. The morphological assessment of human spermatozoa, including the

Table I: Intrauterine insemination pregnancy rate of males with normal sperm parameters.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Primary infertile females (110)</th>
<th>Secondary infertile females (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male partners</td>
<td>Male partners</td>
</tr>
<tr>
<td></td>
<td>females of males of</td>
<td>females of males of</td>
</tr>
<tr>
<td></td>
<td>&lt; 40 (years)</td>
<td>≥ 40 (years)</td>
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<tr>
<td></td>
<td>Total males (94)</td>
<td>Total males (24)</td>
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<td></td>
<td>Male partners</td>
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<td>females of males of</td>
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<tr>
<td></td>
<td>&lt; 40 (years)</td>
<td>≥ 40 (years)</td>
</tr>
<tr>
<td>Normal sperm count (million/ml)</td>
<td>75.78 ± 5.55 (84)</td>
<td>71.5 ± 9.99 (10)</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>Excellent</td>
<td>Good</td>
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<td></td>
<td>30 ± 1.25 (83)</td>
<td>24.87 ± 0.93 (82)</td>
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<td></td>
<td>34 ± 3.05 (10)</td>
<td>26 ± 2.21 (10)</td>
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<tr>
<td></td>
<td>44 ± 3.11**</td>
<td>44 ± 3.11**</td>
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<tr>
<td></td>
<td>27.5 ± 2.5 (20)</td>
<td>27.5 ± 2.5 (20)</td>
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<tr>
<td></td>
<td>22.5 ± 6.29 (4)</td>
<td>22.5 ± 6.29 (4)</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>Normal</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>57.14 ± 0.99 (84)</td>
<td>39 ± 3.48*** (10)</td>
</tr>
<tr>
<td></td>
<td>47.5 ± 3.39 (20)</td>
<td>57.5 ± 4.78 (4)</td>
</tr>
<tr>
<td>Conceived females</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>IUI treatment cycles</td>
<td>172</td>
<td>30</td>
</tr>
<tr>
<td>IUI pregnancy rate %</td>
<td>10.4</td>
<td>30</td>
</tr>
</tbody>
</table>

Values in parentheses indicate the number of male partners of infertile female subjects; **p ≤ 0.01, ***p ≤ 0.001.

Table II: Intrauterine insemination pregnancy rate of males with abnormal sperm parameters.

<table>
<thead>
<tr>
<th>Semen parameters</th>
<th>Total number of males</th>
<th>Sperm count (million/ml)</th>
<th>Mortility (%)</th>
<th>Morphology (%)</th>
<th>IUI treatment cycles</th>
<th>Female conceptions</th>
<th>IUI pregnancy rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Prin</td>
<td>Sec</td>
<td>Prin</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>12</td>
<td>14.58 ± 0.90</td>
<td>24.54 ± 1.57*</td>
<td>40 ± 3.48</td>
<td>15</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Oligoasthenozoospermia</td>
<td>3</td>
<td>10.66 ± 2.40</td>
<td>16.66 ± 3.33</td>
<td>30 ± 5.77</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
<td>3</td>
<td>20.66 ± 5.20</td>
<td>13.33 ± 3.33</td>
<td>46.66 ± 3.33</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*<p ≤ 0.05
evaluation of percentage of morphologically normal sperm and determination of the incidence of various morphological abnormalities has been part of semen analysis consulting for infertility. The present investigation showed a significantly low (p<0.001) normal morphology in male partners of primary infertile females of ≥ 40 years of age compared to male partners of primary infertile females of <40 years of age.

Auger et al. indicated that the detailed assessment of sperm abnormalities is a useful biomarker of the effect of various external factors which may qualitatively affect human spermatogenesis. Defective sperm function has been identified as the greatest among the defined causes of male infertility, accounting for about 27% of all couples attending infertility clinics. The present study showed 12 (8.82%) oligozoospermic, 3 (2.20%) oligoasthenozoospermic and 3 (2.20%) asthenozoospermic males. Poor sperm motility is one of the important causes of male infertility. A significantly high (p<0.05) good grade motility was observed in oligozoospermic males compared to asthenozoospermic males in the present study. Sperm concentration is a strong predictor of fertility in normal male. The present study showed no difference in the sperm counts and morphology of sperms in oligozoospermic, asthenozoospermic and oligoasthenozoospermic males.

This study aimed to establish the normal sperm count and sperm motility and morphology on the success rate in CC –IUI cycles in a selected group of infertile couples undergoing IUI treatment cycles. The present study revealed no statistical difference in the number of conceptions in primary and secondary infertile females with male partners having a normal sperm count. A strong association was seen between the number of IUI cycles and the pregnancy rate (p= 0.0265) of primary and secondary infertile females with male partners having a normal sperm count whereas in a study conducted by Noujua-Huttunen et al., sperm concentration, progressive motility were not predictive of IUI success.

In the present study, IUI pregnancy rate in primary infertile females having oligozoospermic males partners was 20% and secondary infertile females of oligozoospermic males showed 33.3% IUI pregnancy rate. A study was carried out by Tomlinson et al., to find out the IUI pregnancy rate in females with males having severe oligozoospermia and progressive motility showed significantly poor outcome with 0 and 3% pregnancy rates. In the present study, no conceptions were seen in females of oligoasthenozoospermic and asthenozoospermic partners, which is in accordance with Tomlinson et al. who suggested that IUI is highly inappropriate for men with sluggish sperm motility. In a study done by Campana et al., couples in whom the male displayed severe asthenozoospermia or a combination of oligozoospermia and asthenozoospermia with a total motile count of <1x10^6 (million/ml) spermatozoa failed to obtain a pregnancy.

Taking into account the disadvantages of a retrospective study, our results still point towards the importance of performing a well-organized prospective study on IUI success.

**CONCLUSION**

Considering the increased demand for cost benefit analysis and taking into account the very low complication rate of CC-IUI treatment cycles, CC-IUI can be offered to sub-fertile couples. Careful monitoring minimizes the risk for complications like multiple pregnancies and ovarian hyper stimulation. Careful selection criteria coupled with successful ovarian stimulation, therefore, appears to be the model for IUI success. Favourable female characteristics for treatment success are age < 40 years, duration of infertility ≤ 6 years and a cause of sub-fertility other than endometriosis. Regarding males, IUI could be offered to males diagnosed with oligozoospermia, asthenozoospermia and teratozoospermia. Considering the increasing demand for cost benefit-analysis and the very low complication rate, CC-IUI can be offered as the first line treatment to infertile couples.

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