A randomized controlled trial comparing the effectiveness of single versus double intrauterine insemination in unexplained infertility

In a prospective randomized trial comparing efficacy of double over single intrauterine insemination (IUI), 160 couples with unexplained infertility underwent controlled ovarian hyperstimulation and IUI. The pregnancy rates were similar and statistically not different between groups, suggesting that double IUI added no advantage over single IUI. (Fertil Steril 2010;94:2913–5. ©2010 by American Society for Reproductive Medicine.)

Key Words: Unexplained infertility, IUI, single insemination, double insemination

Stimulated intrauterine insemination (IUI) is the first-line treatment for infertility resulting from anovulation, male factor, mild or minimal endometriosis, and unexplained infertility. Despite being less invasive, stressful, or expensive than other assisted reproductive technologies (1), it is one of the least standardized treatments.

There is no consensus in the literature as to whether a double IUI in the same cycle improves pregnancy rate. Although some studies (2, 3) showed improved pregnancy rate with double IUI, other studies (4, 5) suggested that double IUI added no advantage over single. These studies had been limited by heterogeneity of protocols for ovulation induction, semen preparation techniques, and including subjects recruited with possible diagnosis as mild/minimal endometriosis, mild endometriosis, anovulation, mild male factors, and unexplained infertility. A recent meta-analysis on double versus single insemination for unexplained infertility found no clear benefit in overall clinical pregnancy rates (6). More studies are therefore needed to ascertain the possible benefits of performing two inseminations per cycle of controlled ovarian hyperstimulation (COH) and IUI. The present study was therefore planned to compare the efficacy of single and double insemination in a homogeneous group including couples with unexplained infertility with a similar ovarian stimulation and semen preparation protocol.

One hundred sixty couples with unexplained infertility attending the infertility clinics of All India Institute of Medical Sciences, New Delhi, India, were recruited in a prospective randomized study. The study had ethical approval of the Institutional Review Board, and each of the participating couples gave informed consent for the study. The diagnosis of unexplained infertility was made when husband semen analysis according to World Health Organization criteria (7), early follicular phase hormonal assay (FSH, LH, TSH, PRL), hysterosalpingography, and diagnostic laparoscopy and hysteroscopy were normal. Endometrial biopsy was done in all women to exclude genital tuberculosis with conventional and molecular methods (Mycobacterium tuberculosis polymerase chain reaction), because it is an important cause of infertility in this part of the world (8). Women with PCOS, anovulatory infertility, tubal factor infertility, or mild/minimal endometriosis and men with sperm count <20 million/mL were excluded from the study.

All participating women received COH with clomiphene citrate beginning with 50 mg once daily starting from day 3 of cycle for 5 days. In those with no response to 50 mg, the dose of clomiphene citrate was escalated in subsequent cycles for a maximum of 150 mg once daily. Follicle growth was monitored by serial transvaginal sonography beginning day 10, until a dominant follicle of ≥18 mm was attained, when injection of 5,000 U hCG (Profasi; Serum Institute of India, Pune, India) was given intramuscularly. IUI was performed subsequently according to a computer-generated randomization table which defined the group to which the patient was allocated: group A underwent single insemination 34 hours after hCG injection; group B underwent double insemination, 12 hours and 34 hours after hCG injection. There was no cross-over after randomization in subsequent cycles. A maximum of four cycles was offered to couples with unexplained infertility before considering them for IVF, following unit policy.

Semen preparation was done by density centrifugation method. Liquefied semen sample was layered over 2 mL of discontinuous

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two-layer (45% and 90%) gradient solution (Cryo 45% and 90%; Infertech, Delhi, India) taken in a sterile tube. It was centrifuged at 500g for 15 minutes. Seminal plasma, interphase, and Cryo layer were removed carefully, and sperm cells in the 90% solution were transferred and washed in Cryo-HTF solution (HEPES-buffered human tubal fluid; Infertech) by centrifugation at 300g for 5 minutes. The final pellet was suspended in Cryo-HTF solution and incubated at 37°C for 15 minutes. Finally 0.5 mL of the suspension was loaded in a flexible intrauterine catheter (Infertech) and inseminated by introducing the catheter through the cervix using all aseptic precautions. Women were allowed to rest for 20 minutes after the IUI. No luteal support was given after the IUI. Serum β-hCG assay was done if the patient missed a period 16–18 days after the final insemination. Pregnancy was confirmed at 6-weeks ultrasound, and a clinical pregnancy was noted by the presence of fetal heart activity.

Data analysis was performed using SPSS 11.5 (SPSS, Chicago, IL, USA). Results were analyzed by χ² test for comparing pregnancy rate per couple and per cycle. Mann-Whitney U test and Student t test were used for baseline patient characteristics. Paired t test was used for comparing first and second semen sample in the double-IUI group. A P value of <.05 was taken as significant.

Of the 160 randomized patients, 79 underwent 195 cycles of single IUI (group A) and 81 patients underwent 204 cycles of double IUI. The mean duration of infertility was 4.45 years, and the mean age of the female partner was 27.9 years. There were no statistically significant differences between the two groups regarding demographic profile and cycle characteristics (Table 1). There was a significant fall (P<.005) in sperm count between the 12th hour (43.8 ± 24.8 million/mL) and 34th hour (33.1 ± 23.1 million/mL) in the double-IUI group; however, there was no significant difference (P=.16) regarding sperm motility between the 12-hour (55 ± 12%) and 34-hour (53 ± 12%) samples.

Fifty patients from both groups conceived. Overall pregnancy rate per couple was 31.25% and pregnancy rate per cycle 12.8%. Pregnancy rates per couple and per cycle were slightly though not significantly higher in the single-IUI group compared with double-IUI group (Table 1). Of the 50 pregnancies, five (three in Group A and two in Group B) resulted in spontaneous miscarriage, with an overall live birth rate of 88.5% and 87.5% in Group A and group B, respectively, which was statistically nonsignificant. There was one ectopic pregnancy resulting in the double-IUI group, which was managed medically with methotrexate.

Controlled ovarian hyperstimulation with IUI is the first choice of treatment for couples with unexplained infertility. Meta-analysis by Zeyneloglu et al. (9) supports it with better success than COH or IUI alone in unexplained infertility. In our study, overall pregnancy rates per couple and per cycle were 31.25% and 12.8%, respectively, which compares favorably with pregnancy rates reported in the available literature (4, 10). Controversy continues on the number of IUIs needed to improve pregnancy rates, with reported randomized trials comparing single- and double-IUI regimens giving conflicting results. In the present study, pregnancy rate per couple with single insemination (32.9%) was not statistically higher than that with double insemination (29.6%). Our results agree with those of Alborzi et al. (4), but are contrary to those of Silverberg et al. (2) and Ragni et al. (3), who found significantly more pregnancy rates in double-IUI cycles. Double IUI may have been beneficial in the study by Silverberg et al. (2), because one-fourth of the cycle’s utilized cryopreserved semen—which is known to have lower pregnancy rates compared with fresh semen—explaining the value of double IUI. Liu et al. (5), in a randomized study, reported significantly a higher pregnancy rate with double IUI for male factor infertility but no significant increase in couples with unexplained infertility.

**TABLE 1**

Comparison of demographic data and intrauterine insemination outcomes of the study groups between single (group A) and double (group B) insemination.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A (n = 79/195)</th>
<th>Group B (n = 81/204)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of female partner (y)</strong></td>
<td>28.3 ± 3.3</td>
<td>27.2 ± 3.5</td>
<td>.12a</td>
</tr>
<tr>
<td><strong>Duration of infertility (y)</strong></td>
<td>4.5 ± 1.1</td>
<td>4.4 ± 1.3</td>
<td>.59p</td>
</tr>
<tr>
<td><strong>Follicular size (mm)</strong></td>
<td>20 ± 1.5</td>
<td>19.4 ± 1.4</td>
<td>.17a</td>
</tr>
<tr>
<td><strong>No. of follicles</strong></td>
<td>1.8 ± 0.6</td>
<td>1.7 ± 0.5</td>
<td>.52b</td>
</tr>
<tr>
<td><strong>Endometrial thickness (mm)</strong></td>
<td>8 ± 0.8</td>
<td>7.6 ± 0.6</td>
<td>.29a</td>
</tr>
<tr>
<td><strong>Prewash sperm count (million/mL)</strong></td>
<td>66.6 ± 33.2</td>
<td>49.3 ± 24.8</td>
<td>.77a</td>
</tr>
<tr>
<td><strong>Postwash sperm count (million/mL)</strong></td>
<td>37.6 ± 21.5</td>
<td>27.1 ± 19.5</td>
<td>.79a</td>
</tr>
<tr>
<td><strong>Sperm motility (%)</strong></td>
<td>59.8 ± 8.3</td>
<td>53.6 ± 11.4</td>
<td>.17p</td>
</tr>
<tr>
<td><strong>Pregnancy rate (per cycle, %)</strong></td>
<td>13.3%</td>
<td>11.8%</td>
<td>.63c</td>
</tr>
<tr>
<td><strong>Pregnancy rate (per couple, %)</strong></td>
<td>32.9%</td>
<td>29.6%</td>
<td>.65c</td>
</tr>
<tr>
<td><strong>Miscarriage rate (miscarriage/overall pregnancy, %)</strong></td>
<td>11.5%</td>
<td>8.3%</td>
<td>.53c</td>
</tr>
<tr>
<td><strong>Ectopic pregnancies (ectopic pregnancy/overall pregnancy) (%)</strong></td>
<td>0</td>
<td>1/24 (4.2%)</td>
<td>.5c</td>
</tr>
<tr>
<td><strong>Delivery rates (%)</strong></td>
<td>23/26 (88.5%)</td>
<td>21/24 (87.5%)</td>
<td>.5c</td>
</tr>
</tbody>
</table>

*Note: Data are presented as mean ± SD.*

*Student t test.*

*Mann-Whitney U test.*

*Chi-square test.*

Advocates of double IUI suggest that increasing the number of inseminations might improve the chance of conception because of the unsynchronized ovulation pattern in COH with multiple ovulations sequentially over ≥24 hours. Although first insemination would provide sufficient spermatozoa before the first released oocyte, the second insemination would provide additional spermatozoa to fertilize oocytes likely to be released subsequently (3). Animal studies demonstrate that, <10% of inseminated sperm are retained in the upper reproductive tract even 12 hours after IUI (11). This attrition phenomenon, along with observations showing a reduced sperm motility, further adds appeal to double IUI (12). Another explanation for higher conception after double IUI is that patients receiving two inseminations had a significantly greater number of spermatozoa inseminated, which may be an important factor (3). Hornstein et al. (13) observed significant decrease in semen volume, sperm counts, and motility in the second consecutive day semen sample; however, there was no significant difference in sperm motility but a significant decline in sperm count in the second sample in the double-IUI group in our study. However, the pregnancy rates did not differ significantly. The impact of this decline is not clear, particularly when an insemination of as little as 1 million motile sperm is reported to be necessary to achieve pregnancy (14).

Factors determining success of COH-IUI include age of the female partner, duration of infertility, number of developing follicles, endometrial thickness, and post–semen preparation sperm motility and concentration (15, 16). In the present study, there was no significant difference regarding these parameters affecting the outcome between the groups. We focused mainly on the number of inseminations and found no difference in that aspect.

To conclude, our data does not support the hypothesis that doubling the number of inseminations significantly improves the pregnancy rate over a single well timed IUI. We therefore suggest that a single well timed IUI is sufficient in patients undergoing COH-IUI for unexplained infertility.

REFERENCES