Correct timing of intrauterine insemination (IUI) might optimize the success rate in fertile couples (1). Nonetheless, timing of ovulation varies and might depend on the ovarian stimulation protocol used (2). A way to bypass this problem in IUI cycles is to increase the frequency of IUI in the same treatment cycle. Early randomized trials revealed higher clinical pregnancy rates during double IUI treatment cycles (3). However, it is unclear whether increasing IUI frequency per treatment cycle might benefit couples with unexplained infertility.

Women with unexplained infertility are a group of patients who might not experience substantial benefits from the use of IUI compared with women with other infertility factors. Recently published randomized trials (4, 5) have seriously questioned the effect of IUI with or without controlled ovarian hyperstimulation (COH) compared with 6-months-expectant management in these women.

We therefore set to perform a meta-analysis of randomized trials, in order to investigate whether double IUI with a partner’s sperm can enhance IUI success rates in couples with unexplained infertility.

MATERIALS AND METHODS
Identification of Randomized Studies
Two independent investigators (D.M., S.T.) searched the Cochrane Central Trials Registry and Medline without year and language restriction through March 2009; hand searching of the abstract books of the European Society of Human Reproduction and Embryology and American Society for Reproductive Medicine annual meetings (2001–2008). Six randomized trials, involving 829 women, were included in the analysis. Fifty-four (13.6%) clinical pregnancies were recorded for treatment with double IUI and 62 (14.4%) for treatment with single IUI. There was no significant difference between the single and double IUI groups in the probability for clinical pregnancy (odds ratio, 0.92; 95% confidence interval, 0.58–1.45; P=0.715).

Conclusion(s): Double IUI offers no clear benefit in the overall clinical pregnancy rate in couples with unexplained infertility. (Fertil Steril 2010;94:1261–6. ©2010 by American Society for Reproductive Medicine.)

Key Words: Intrauterine insemination, IUI, double IUI, unexplained infertility, meta-analysis
systematically differed in the use of these ovarian stimulation agents were excluded, because the differences in pregnancy rates and additional outcomes could not have been necessarily attributed to the contrast of single vs. double IUI per cycle treatment.

Data Extraction
Data were extracted by two independent investigators (N.P.P., S.T.) and potential discrepancies were resolved with the involvement of a third investigator (D.M.). From each eligible trial we recorded for both arms the following items: authors’ names, journal and year of publication, country of origin, number of patients with unexplained infertility randomized per arm, number of cycles per arm, infertility duration, mean age at enrollment, type of COH used, type of sperm preparation technique, and the exact timing of single and double inseminations per cycle. Finally, we recorded study design items, including whether there was a description of the mode of randomization, allocation concealment, and blinding.

Analysis
Our analysis addressed the odds ratio (OR) for the clinical pregnancy per couple for the comparison of double vs. single IUI for unexplained infertility. For each eligible study group, we estimated the OR for clinical pregnancy between the groups in comparison and the 95% confidence interval (CI). Between-study heterogeneity for the OR was evaluated using the Q statistic (7, 8). We then synthesized the data across studies using both fixed effects (Mantel–Haenszel) and random effects (DerSimonian and Laird) modeling (8). Analyses were performed in STATA SE 10.0 (Stata Corp., College Station, TX). All $P$ values are two-tailed.

RESULTS

Eligible Trials
The electronic search yielded 1,722 items; 1,128 from Medline and 594 from Cochrane Library. All eligible articles were written in English. Twenty-eight reports were scrutinized in full text, 18 were excluded and 2 trials were retrieved from hand-searching of references, ASRM and ESHRE abstract books. Twelve randomized trials were considered potentially eligible and met our inclusion criteria. However, five of them were disqualified for lack of separate data for couples with unexplained infertility. One additional study (3) provided information only on the number of cycles per arm and was excluded from our analysis. (Fig. 1).

Finally, six randomized trials with data regarding the pregnancy rate per couple were considered eligible (Table 1). All six eligible randomized trials involved patients receiving homologous IUI with partners’ sperm and were published between 1997 and 2007. Cumulatively, 829 women with a mean age ranging 27.4–35 years were randomized to either single or double IUI per treatment cycle.

In all of the trials, the ovulation triggering was achieved by intramuscular injection of hCG with doses of 5,000 or 10,000 IU. The ovarian stimulation protocol varied among included trials; one trial used only clomiphene (9), three trials only gonadotrophins (10, 11, 12), and two trials a combination of clomiphene and gonadotrophins (13, 14).

The timing of single IUI was performed around the time of ovulation (approximately 36 hours after hCG administration) in most of the trials; in only one trial (12) was single IUI performed earlier in the preovulatory period (24 hours after hCG administration). Timing of first and second IUI in double IUI cycles was also similar in all of the trials included, with the former performed in the preovulatory period (12–24 hours after hCG administration) and the latter in the periovulatory period (34–48 hours after hCG administration).

Two different sperm preparation techniques were used in the eligible trials. Four trials used the density gradient technique (9, 11–13), and two trials (10, 14) used the swim-up technique.

Quality Assessment of Trials
Five of six trials (9–11, 13, 14) (83%) provided a sufficient randomization mode. One trial ensured allocation concealment (10), whereas none were blinded. Finally, one trial (14) was a crossover trial; however, data regarding pregnancies were available from the first treatment cycle (prior cross-over) and the trial was included in the analysis (Table 1).

Meta-analysis
Six randomized trials including 829 patients were combined (Table 2). After double IUI, 54 (13.6%) clinical pregnancies were observed; however, after single IUI, there were 62 (14.4%) clinical pregnancies. Both random and fixed effects model yielded similar results (OR, 0.92; 95% CI, 0.59–1.45; $P=0.715$ for random effects model; OR, 0.93; 95% CI, 0.62–1.40, $P=0.723$ for fixed effects model; Fig. 2). There was no significant difference in the probability to have a pregnancy in women after double IUI as compared to women with single IUI. No significant between-study heterogeneity ($Q = 5.36; P=0.373$) was detected. The result remained nonsignificant when two studies (9, 12), which were published as abstracts...
# TABLE 1

Baseline characteristics of eligible trials.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Country</th>
<th>Journal</th>
<th>Timing of IUI (h)</th>
<th>Age (y)</th>
<th>Infertility Duration (y)</th>
<th>Ovarian stimulation protocol</th>
<th>Sperm preparation technique</th>
<th>Methodological quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malhorta (2007)</td>
<td>India</td>
<td>Fertil Steril</td>
<td>DIUI-1st 12&lt;sup&gt;a&lt;/sup&gt; 2nd 34&lt;sup&gt;a&lt;/sup&gt; SIUI-34</td>
<td>ND</td>
<td>ND</td>
<td>1) CC 50-150 mg for 5 d 2) hCG</td>
<td>Density gradient method</td>
<td>Random mode: Yes Allocation concealment: No Blinding: No Cross over: No</td>
</tr>
<tr>
<td>Liu (2006)</td>
<td>China</td>
<td>J Assist Reprod Genet</td>
<td>DIUI-1st 18-24 2nd 36-48 SIUI-34</td>
<td>34.9</td>
<td>4.2</td>
<td>1) CC 50 mg/d for 5 d 2) HMG 75-150 IU/d 3) hCG 10000 IU</td>
<td>Density gradient method (Percoll)</td>
<td>Random mode: Yes Allocation concealment: No Blinding: No Cross over: No</td>
</tr>
<tr>
<td>Albrozi (2003)</td>
<td>Iran</td>
<td>Fert Steril</td>
<td>DIUI-1st 12 2nd 34 SIUI-34</td>
<td>27.4</td>
<td>5.2</td>
<td>1) CC 100 mg for 5 d 2) hMG 150 IU/d 3) hCG 5000-10000 IU</td>
<td>Swim-up</td>
<td>Random mode: Yes Allocation concealment: No Blinding: No Cross over: Yes</td>
</tr>
<tr>
<td>Ng (2003)</td>
<td>China</td>
<td>J Reprod Med</td>
<td>DIUI-1st 18 2nd 42 SIUI-38</td>
<td>32.9 (DIUI) 32.7 (SIUI)</td>
<td>&gt;2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1) HMG 150 IU/d 2) hCG 10000 IU</td>
<td>Density gradient method (Isolate)</td>
<td>Random mode: Yes Allocation concealment: No Blinding: No Cross over: Yes</td>
</tr>
<tr>
<td>Peddie (1997)</td>
<td>UK</td>
<td>J Reprod Fertil</td>
<td>DIUI-1st 24 2nd 48 SIUI-24</td>
<td>ND</td>
<td>&gt;4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1) HMG 2) hCG 10000 IU</td>
<td>Density gradient method (Percoll)</td>
<td>Random mode: No Allocation concealment: No Blinding: No Cross over: No</td>
</tr>
</tbody>
</table>

Note: IUI = Intrauterine insemination; h = hours after administration of hCG; ND = no data provided; DIUI = double intrauterine insemination; SIUI = single intrauterine insemination; CC = clomiphene citrate.

<sup>a</sup> 1st<sup>st</sup> and 2nd<sup>nd</sup> refers to the 1<sup>st</sup> and 2<sup>nd</sup> IUIs performed in double IUI treatment cycles.

<sup>b</sup> Value represents the least duration of infertility for inclusion in the trial.

TABLE 2

Number of patients, cycles, and pregnancies per treatment arm.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Treatment</th>
<th>Patients (n)</th>
<th>Treatment cycles (n)</th>
<th>Pregnancy (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malhorta (2007)</td>
<td>Double IUI</td>
<td>38</td>
<td>112</td>
<td>13</td>
</tr>
<tr>
<td>Liu (2006)</td>
<td>Single IUI</td>
<td>34</td>
<td>96</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Double IUI</td>
<td>243</td>
<td>243</td>
<td>29</td>
</tr>
<tr>
<td>Casadei (2006)a</td>
<td>Single IUI</td>
<td>247</td>
<td>247</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Double IUI</td>
<td>10</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Alborz (2003)</td>
<td>Single IUI</td>
<td>24</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Double IUI</td>
<td>28</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>Ng (2003)a</td>
<td>Single IUI</td>
<td>31</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>Peddie (1997)a</td>
<td>Single IUI</td>
<td>7</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Double IUI</td>
<td>75</td>
<td>ND</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>Single IUI</td>
<td>88</td>
<td>ND</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Double IUI</td>
<td>398</td>
<td>—</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Single IUI</td>
<td>431</td>
<td>—</td>
<td>62</td>
</tr>
</tbody>
</table>

Note: IUI = intrauterine insemination; ND = no data available.

a Data obtained after personal contact with the primary investigators of the trials.


Our meta-analysis provides evidence that double IUI does not result in higher pregnancy rates compared with single IUI treatment in women with unexplained infertility undergoing COH with clomiphene or gonadotrophins. Whereas it is suggested that double IUI may deliver more spermatozoa to the site of fertilization and fertilize more oocytes because multiple ovulations during stimulated cycles are sequential over a period of at least several hours (15, 16), we could not find any difference in the clinical pregnancy rate compared with traditional treatment with a single IUI per cycle. This finding could be attributed to the fact that the benefit of a “wider fertilization window” might not be adequate to increase clinical pregnancy rates in women with unexplained infertility. Intrauterine insemination with or without COH does not appear to offer a clear benefit compared with 6-months-expectant management in women with no specific cause of infertility. Because IUI does not offer a clear benefit in couples with unexplained infertility, increasing the frequency of IUI might have a detrimental effect on the clinical pregnancy rates in this subgroup of patients when compared with women with other causes of infertility.

Two previous systematic reviews assessed the effect of double IUI per treatment cycle in women with different causes of infertility. Whereas the first revealed a trend (6) and the second a statistically significant benefit for the treatment with double IUI per treatment cycle (17), in both systematic reviews, eligible trials considerably differed regarding the indication of treatment and included patients with different causes of infertility (male factor, cervical, and unexplained infertility). However, it is well established that the type of infertility is strongly correlated with live birth and clinical pregnancy rates in IUI cycles (18, 19), and therefore estimating the effect of a treatment modality in women with different infertility indications may result in biased or flawed results.

One of the strengths of our meta-analysis is that we followed the most rigorous methodology for performing systematic reviews to eliminate potential biases. According to our protocol, we included in our analysis trials that have been published in meeting abstracts (9, 12) in an attempt to eliminate the likelihood of publication bias. Sensitivity analysis performed by excluding these trials did not change the results, and no difference was observed in favor of any of the compared arms. Furthermore, we included in our analysis a cross-over trial (14). In cross-over trials, the treatment evaluation becomes uncontrolled, unless the analysis is restricted to the first period before the cross-over point, in which case the trial has a parallel design but with inadequate power because the sample size is insufficient (20, 21). Therefore, we included in our analysis only data from the first treatment cycle before cross-over. Finally, we excluded one trial because the authors provided data regarding pregnancy rates only per cycle and not per couple (3), and any attempt to retrieve additional information for this trial failed. We decided not to perform a meta-analysis for pregnancy rates per treatment cycle, because the lack of independence among cycles of the same individual (i.e., between cycle variation) might bias the results.

Another advantage of our meta-analysis is that we limited our analysis to a more homogenous group of patients, including only couples with unexplained infertility as a sole treatment indication. This advantage might be reflected by the lack of between-study-heterogeneity in our meta-analysis compared with both previous reviews. Furthermore, our systematic review was comparatively homogenous because the precise timing of single and double IUI was similar in the trials included. According to our protocol, we excluded arms in which the second IUI was delivered late in the postovulatory period (48 hours after hCG administration). Hence, in all of the trials, single IUI was administrated in the periovulatory period (approximately 36 hours after hCG administration), and in double IUI protocols, the first in the preovulatory and the second in the periovulatory period. In only one trial (12) was the single IUI performed earlier in the preovulatory period (24 hours after hCG administration). In this trial, the clinical pregnancy rate did not differ between compared arms, whereas when we performed a sensitivity analysis by excluding it from our analysis, the difference between the compared arms remained nonsignificant.

However, several parameters that might affect the results differed among eligible trials. The ovarian stimulation protocol used was not similar in all of the trials included. Clomiphene, gonadotrophins, or a combination of both were used to induce ovulation. This might have resulted in different pregnancy rates among analyzed trials. Induction with clomiphene can result in a certain number of oocytes able to be fertilized as early as 12 hours after hCG administration.

Our meta-analysis provides evidence that double IUI does not result in higher pregnancy rates compared with single IUI treatment in women with unexplained infertility undergoing COH with clomiphene or gonadotrophins. Whereas it is suggested that double IUI may deliver more spermatozoa to the site of fertilization and fertilize more oocytes because multiple ovulations during stimulated cycles are sequential over a period of at least several hours (15, 16), we could not find any difference in the clinical pregnancy rate compared with traditional treatment with a single IUI per cycle. This finding could be attributed to the fact that the benefit of a “wider fertilization window” might not be adequate to increase clinical pregnancy rates in women with unexplained infertility. Intrauterine insemination with or without COH does not appear to offer a clear benefit compared with 6-months-expectant management in women with no specific cause of infertility. Because IUI does not offer a clear benefit in women with unexplained infertility, increasing the frequency of IUI might have a detrimental effect on the clinical pregnancy rates in this subgroup of patients when compared with women with other causes of infertility.
Meta-analysis using random and fixed-effects models for live pregnancy rate of double vs. single intrauterine insemination per treatment cycle in women with unexplained infertility. Each study is shown as an odds ratio (OR) estimate (black squares), with whiskers corresponding to 95% confidence intervals (CIs). The size of the squares is proportional to the weight the study contributes to the meta-analysis. Studies are ordered by year of publication. Also shown is the summary OR (black diamond) by fixed effects calculations. Vertical reference lines show the line of no association (continuous line) and the overall odds ratio (discontinuous line).

(22); moreover, in cycles stimulated by gonadotrophins, 24% of them can lead to undesired premature LH surge and subsequently to IUI cycle cancellation (23). Nevertheless, in all the trials, regimens used for ovarian stimulation did not systematically differ among compared arms.

Heterogeneity was also present concerning the sperm preparation techniques used, because some of the trials adopted the density gradient and some adopted the swim-up technique. Nonetheless, currently available evidence does not support any difference among these two sperm preparation techniques in terms of live births, pregnancy rates, or miscarriages (24).

Although several factors might have contributed to heterogeneity among the studies, heterogeneity test was not significant. Because of the small number of studies included in our meta-analysis, we may have not been able to detect heterogeneity among them (25). Consequently, using the random rather than the fixed effects model to present our results would be considered as more appropriate.

A limitation in our meta-analysis is that it is based on published data and not on individual patient data. In the future, a meta-analysis of individual patient data from several infertility centers might be a more appropriate approach. In addition, methodologic parameters were not reported in several of the eligible trials. The need of methodologic quality reporting in infertility trials has been previously underlined (26). Lack of reporting of methodologic parameters has been associated with spuriously inflated treatment effect (27). However, methodologic quality measures of randomized trials do not always affect estimates of treatment effect in all medical domains (28), and blinding is not always convenient in infertility trials.

Considering the strengths and allowing for the limitations discussed, our meta-analysis suggests that double homologous IUI does not result in higher clinical pregnancy compared with a single IUI in couples with unexplained infertility. We cannot preclude that future randomized trials might reveal a significant benefit of double IUI per treatment cycle; nonetheless, this may be more likely to happen in couples that do not have unexplained infertility.

Acknowledgments: We would like to thank Dr. Ernest Ng, Dr. Val Peddie, Dr. Luisa Casadei, Dr. Mark Ransom, and Dr. Mark Hamilton and for data provided from their trials.

REFERENCES