Which is the Best Protocol of Ovarian Stimulation Prior to Artificial Insemination by Donor

Bu-fang XU, Guang-yan WANG, Wei-min FAN, Qian CHEN, Ai-jun ZHANG
Reproductive Medical Center of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Objective  To compare the different ovarian stimulation protocols, clomiphene citrate (CC), letrozole, human menopausal gonadotropin (hMG) only or combined with CC or letrozole in women undergoing artificial insemination by donor (AID).

Methods  In this prospective clinical trial, 671 couples prepared for AID cycles were randomly allocated to 6 groups according to receive different protocols for the first time, natural cycle (group A, n=114), CC (group B, n=101), CC and hMG (group C, n=124), letrozole (group D, n=97), letrozole and hMG (group E, n=123) and hMG only (group F, n=112). Outcomes including total dose of hMG, duration of hMG therapy, dominant follicles number, endometrial thickness, rates of clinical pregnancy, miscarriage, ovarian hyperstimulation syndrome (OHSS), multiple pregnancy and cancelation were compared among the 6 groups.

Results  The total doses and duration of administered hMG were significantly lower in group C and group E than in group F. Dominant follicle number was significantly less in group A and more in group C than in other groups. Endometrial thickness of group B was significantly lower than that of other groups. Clinical pregnancy rate, multiple pregnancy rate, miscarriage rate, OHSS rate and cancelation rate were not statistically different among the stimulation groups.

Conclusion  AID cycles in which both CC and letrozole had been administered may require shorter duration and a lower total gonadotropin dose, while the clinical outcomes were similar.

Key words: intrauterine insemination; clomiphene citrate (CC); letrozole; human menopausal gonadotropin (hMG)

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Corresponding author: Ai-jun ZHANG; E-mail: zhaj1268@163.com
Intrauterine insemination (IUI) can be accomplished in a natural cycle or with ovarian stimulation. There has been little controversy that ovarian stimulation protocol can improve pregnancy rate in IUI compared with natural cycle\(^1\), but till now there is no consensus about which protocol is the first choice for inducing ovulation. In the clinic, clomiphene citrate (CC), letrozole (both can combined with gonadotrophin) and gonadotrophin only are used for ovarian stimulation. Gonadotropin accomplished alone may increase multiple pregnancy rate and ovarian hyperstimulation syndrome (OHSS) rate\(^2,3\). So, taking account of cost, comfort and security, CC and letrozole are widely used for ovulation inducing before IUI, which can increase endogenous follicle stimulating hormone (FSH) by inducing estrogen receptors depletion or suppress the production of estrogen. But the benefit of CC and letrozole used before hMG was debated, in the study of Mahani et al.\(^4\), hMG group had a higher pregnancy rate compared with CC group. But the conclusion of Ibrahim et al.\(^5\) was just the opposite: the pregnancy rate of CC group was higher than that of hMG group.

For more than four decades, CC is a popular choice for inducing ovulation because of effective and cheap. However it induces estrogen receptor depletion and has anti-estrogenic effect on endometrium, which may be the reason of low pregnancy rate. As an aromatase inhibitor, letrozole has no adverse effect on endometrium. It was reported that letrozole was superior to CC for inducing ovulation in IUI in some studies\(^6,7\), but others got the opposite results\(^8-10\). Artificial insemination by donor (AID) is proposed for couples when infertility is established to be due to severe male factors, AID is the special case of IUI which can avoid some male infertility factors. The purpose of our study was to compare the efficacy of different ovary stimulation protocols in women undergoing AID.

Materials & Methods

Patients and groups

This randomized clinical trial was approved by the Ethics Committee of Ruijin Hospital, Medical School of Shanghai Jiao Tong University. Recruitment was conducted in Reproductive Center of Ruijin Hospital from September 2009 to June 2012. All couples had been unable to conceive for at least 1 year before coming to our IVF center for treatment. The reasons for AID were azoospermia or chromosome abnormality on the male’s side. In our study, all females were assessed by hysterosalpingography (HSG) before IUI treatment and had at least one patent tube. Couples were excluded if there were a history of previous assisted reproduction attempts by IUI, IVF or ICSI, women with endometriosis (classification stage III and IV of the American Infertility Society), myoma, endometrial polyps or polycystic ovary syndrome (PCOS)\(^11\), or contraindication to one of the investigated drugs, persistent ovarian cyst (a cyst of at least 30 mm persisting for longer than 2 months) were excluded. Women with normal ovulation was monitored in nature cycle (group A, \(n=114\)), women with
irregular period were randomly allocated to 5 groups, CC (group B, \(n=101\)), CC and hMG (group C, \(n=124\)), letrozole (group D, \(n=97\)), letrozole and hMG (group E, \(n=123\)) and hMG only (group F, \(n=112\)).

All participants underwent a baseline transvaginal ultrasound (Siemens, Sonoline G20) using a 7.5 MH transvaginal probe.

**Ovulation monitoring/stimulation**

The development of follicles was monitored by transvaginal ultrasonography (TVS). Group A: for females with regular menstrual cycle, the monitoring of the development of follicles started on day 10–12 of menstrual cycle. When the diameter of dominant follicle was as large as 15 mm, the patient was asked to monitor the urinary luteinizing hormone (LH) test twice per day. If the urinary LH test was positive or the urinary LH test was negative but the diameter of dominant follicle was larger than 17–18 mm, 5,000 IU of human chorionic gonadotropin (hCG, Lizhu Pharmaceuticals, Zhuhai, China) were given. Then IUI was performed next day. Group B: CC (Cyprusgote Pharmaceuticals, Limassol, Cyprus), in a dose of 50–100 mg/d was only given on day 5–9 of menstrual cycle. The follicular diameter and endometrial thickness were monitored on day 10 of menstrual cycle. If the development of dominant follicle was not good (group C), a low-dose hMG (hMG, Lizhu Pharmaceuticals, Zhuhai, China) was subsequently added from 37.5 IU to 75.0 IU according to female’s body mass index (BMI) and age, then patients came back for monitor repeated or every other day depending on the development of follicles. Group D: letrozole (Jiangsu Hengrui Pharmaceuticals, Lianyungang, China) in a dose of 2.5–5.0 mg/d was given on day 5–9 of menstrual cycle, and the other procedure was the same as group B. If the development of dominant follicle was not good (group E), the procedure was the same as group C. Group F: a low-dose step-up protocol was given. hMG was intramuscular injected in a dose of 37.5–75.0 IU/d for 4 d starting on day 3–5 of menstrual cycle. The follicular diameter and endometrial thickness were also monitored on day 5 after hMG administration. Then the dose of hMG would be adjusted according to the development of dominant follicles if needed. In above-mentioned five ovarian stimulation groups, when the diameter of one or more dominant follicles was larger than 17–18 mm, 5,000 IU of hCG was scheduled for intramuscular injection, endometrial thickness and the number of dominant follicles (≥16 mm in diameter) was recorded. IUI was done on next day after hCG administration as same as nature group. When the dominant follicles were more than 4, or endometrial thickness was less than 7 mm on the day before IUI, this cycle was canceled.

**Sperm preparation and IUI procedure**

Frozen sperm, purchased from human sperm bank, was thawed routinely and then prepared in density gradient centrifugation technology before IUI. IUI was performed with a catheter in a routine way with women in lithotomy position. Insemination volumes ranged from 0.3 ml to 0.5 ml. After IUI performed, women were asked to lie flat at least half an hour.
Pregnancy confirmation

Plasma β-hCG levels were measured 2 weeks after IUI. Chemical pregnancy was confirmed when β-hCG level >5 IU/L. Clinical pregnancy was confirmed by the presence of an intrauterine gestation sac on TVS. Multifetal pregnancy was defined as two or more intrauterine sacs on TVS. Miscarriage was defined as pregnancy loss before 12 weeks.

Statistical analysis

Statistical analysis was performed using IBM SPSS for Windows, Version 19.0 (Armonk, NY: IBM Corp.). Continuous variables were expressed as mean ± standard deviation (x ± s). The Χ² test was used to compare proportions of each group, and ANOVA analysis was used to compare mean of each group. A P value <0.05 was considered significant.

Results

A total of 671 couples enrolled in the present study and were divided into 6 groups. Twenty-seven couples were unable to complete the total course of treatment. The mean age were 28.8 ± 3.5 (20–39) years, and BMI was 21.3 ± 5.8 (15.6–34.9) kg/m² of all female, the differences among 6 groups had no significance. Demographic data were comparable in 6 groups and are summarized in Table 1.

Table 1  Baseline characteristics of six groups (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Female age (year)</th>
<th>BMI (kg/m²)</th>
<th>Infertility duration (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>114</td>
<td>28.9 ± 3.8</td>
<td>20.5 ± 2.3</td>
<td>2.9 ± 1.0</td>
</tr>
<tr>
<td>B</td>
<td>101</td>
<td>29.2 ± 3.6</td>
<td>20.9 ± 2.9</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>C</td>
<td>124</td>
<td>28.0 ± 3.4</td>
<td>21.7 ± 3.6</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>D</td>
<td>97</td>
<td>29.0 ± 4.5</td>
<td>21.0 ± 2.5</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>E</td>
<td>123</td>
<td>28.2 ± 3.0</td>
<td>21.8 ± 2.9</td>
<td>3.6 ± 1.1</td>
</tr>
<tr>
<td>F</td>
<td>112</td>
<td>29.0 ± 3.5</td>
<td>21.3 ± 3.0</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Duration of hMG therapy was significantly shorter in both group C and group E than in group F (4.2 ± 0.7 d and 4.9 ± 0.8 d vs 6.9 ± 2.3 d, P<0.05). Total dose of administered hMG was also significantly lower in both group C and group E than in group F (285.0 ± 172.5 IU and 292.5 ± 202.5 IU vs 502.5 ± 285.0 IU, P<0.05). The number of dominant follicles (≥16 mm) was significantly more in all ovary stimulation groups than in group A, and was the most in group C (1.8 ± 0.9) significantly. The endometrial thickness was significantly lower in group B than in other groups, while when CC administered with hMG (group C), the endometrium was much thicker (Table 2).

Sixteen (14.0%) pregnancies observed in group A was lower than which in all the stimulation groups, this difference had statistical significance (P<0.05), while there was
no significant difference among the stimulation groups. The multiple pregnancy rates were significantly a little higher in group E and group F than in group A (5.7% and 21.7% vs 0.0%, \( P < 0.05 \)). There was no significant difference of biochemical pregnancy and first trimester spontaneous abortion rates among all the groups. OHSS in group C and group E were all mild to moderate, and none needed inpatient management. The OHSS rate was higher in group C and group F than in other 4 groups (4.8% and 3.6% vs 0.0%, \( P < 0.05 \)). Five (4.4%) women in group A were canceled because of abnormal ovulation, 2 (2.0%) women of group B were canceled because of thin endometrium, a total of 12 (9.7%) women of group C were canceled, 2 because of mild OHSS, 10 because of thin endometrium. One was canceled in group E because of thin endometrium. Seven (6.2%) women were canceled in group F, 4 because of mild OHSS and 3 because of thin endometrium, no women was canceled in group D. The difference of all the groups had no significance (Table 3).

**Discussion**

AID is a good model for study ovarian stimulation protocols in IUI because of no male

### Table 2  Cycle characteristics in six groups (\( \bar{x} \pm s \))

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Total dose of hMG (IU)</th>
<th>Duration of hMG therapy (d)</th>
<th>Number of dominant follicles ( \geq 16 ) mm on the day before IUI</th>
<th>Endometrial thickness on day before IUI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>114</td>
<td>/</td>
<td>/</td>
<td>1.0 ( \pm 0.1 )</td>
<td>9.5 ( \pm 1.5 )</td>
</tr>
<tr>
<td>B</td>
<td>101</td>
<td>/</td>
<td>/</td>
<td>1.4 ( \pm 0.6 )</td>
<td>8.5 ( \pm 1.5 )</td>
</tr>
<tr>
<td>C</td>
<td>124</td>
<td>285.0 ( \pm 172.5 )</td>
<td>4.2 ( \pm 0.7 )</td>
<td>1.8 ( \pm 0.9 )</td>
<td>9.2 ( \pm 1.6 )</td>
</tr>
<tr>
<td>D</td>
<td>97</td>
<td>/</td>
<td>/</td>
<td>1.3 ( \pm 0.4 )</td>
<td>8.9 ( \pm 1.5 )</td>
</tr>
<tr>
<td>E</td>
<td>123</td>
<td>292.5 ( \pm 202.5 )</td>
<td>4.9 ( \pm 0.8 )</td>
<td>1.4 ( \pm 0.7 )</td>
<td>9.5 ( \pm 2.3 )</td>
</tr>
<tr>
<td>F</td>
<td>112</td>
<td>502.5 ( \pm 285 )</td>
<td>6.9 ( \pm 2.3 )</td>
<td>1.3 ( \pm 0.6 )</td>
<td>9.4 ( \pm 1.8 )</td>
</tr>
</tbody>
</table>

a: \( P < 0.05 \), compared with group F  
b: \( P < 0.05 \), compared with group A  
c: \( P < 0.05 \), compared with group B  
d: \( P < 0.05 \), compared with group C

### Table 3  Pregnancy outcomes in six groups (\( \bar{x} \pm s \))

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Clinical pregnancy rate (( n ))</th>
<th>Miscarriage rate (( n ))</th>
<th>Multiple pregnancy rate (( n ))</th>
<th>OHSS rate (( n ))</th>
<th>Cancelation rate (( n ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>114</td>
<td>14.0% (16)</td>
<td>2.5% (2)</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
<td>4.4% (5)</td>
</tr>
<tr>
<td>B</td>
<td>101</td>
<td>20.8% (21)</td>
<td>19.0% (4)</td>
<td>14.3% (3)</td>
<td>0.0% (0)</td>
<td>2.0% (2)</td>
</tr>
<tr>
<td>C</td>
<td>124</td>
<td>20.2% (25)</td>
<td>12.0% (3)</td>
<td>16.0% (4)</td>
<td>4.8% (6)</td>
<td>9.7% (12)</td>
</tr>
<tr>
<td>D</td>
<td>97</td>
<td>18.6% (18)</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
</tr>
<tr>
<td>E</td>
<td>123</td>
<td>28.5% (35)</td>
<td>5.7% (2)</td>
<td>5.7% (2)</td>
<td>0.0% (0)</td>
<td>0.8% (1)</td>
</tr>
<tr>
<td>F</td>
<td>112</td>
<td>20.5% (23)</td>
<td>8.7% (2)</td>
<td>21.7% (5)</td>
<td>3.6% (4)</td>
<td>6.2% (7)</td>
</tr>
</tbody>
</table>

b: \( P < 0.05 \), compared with group A  
c: \( P < 0.05 \), compared with group B  
e: \( P < 0.05 \), compared with group D  
f: \( P < 0.05 \), compared with group E
infertility factors, and in this study, the women had at least one patent tube. So it is accurate to compare different stimulation protocols in women accepted AID.

There has been little controversy that ovarian stimulation can improve pregnancy rate in IUI compared with natural cycle. It was reported that hyperstimulation was benefit for persistent infertility in IUI, and compared with natural cycles, the pregnancy rate was similar but multiple pregnancy rates were much higher, also the live birth rate was increased[12-14]. In this present study, the clinical pregnancy rates of all stimulation groups were significantly higher than nature group, which was in agreement with data from literatures. There were two reasons, firstly, the document follicles on the day before IUI of all stimulation groups were significantly more than which of nature group, which might increase the embryo number. Secondly, we thought the endometrial receptivity was not damaged in stimulation groups at least. The endometrial thickness of letrozole group, letrozole and hMG group, and hMG only group were similar to which of nature group, and although the endometrium was a little thicker in CC group and CC and hMG group, it was also enough for embryos implantation (>8 mm)[15].

It was reported that hMG promoting follicle development might increase the rate of multiple pregnancy and OHSS[3]. But in the hMG group in this study, there was no moderate and severe OHSS, the mild OHSS rate (2.3%) was acceptable, and the difference in term of multiple pregnancy rates (2.9%) between hMG group and other groups had no significance. The reason we thought was that the dose of hMG in stimulation groups was low, from 37.5 IU to 75.0 IU per day, which was safe and effective for stimulation ovulation.

CC has been widely used for inducing ovulation for several decades because of cheap and efficiency, however as an estrogen receptor antagonist, it might decrease pregnancy rate and increase miscarriage rate by affecting the endometrial receptivity[6,11,16]. But in this clinical study, only 2.0% (2/101) women were canceled because of thin endometrium in CC group, and the average endometrial thickness was a little lower than other groups, meanwhile the clinical pregnancy rate of CC group was similar to other stimulation groups, so the side effect of CC to endometrial receptivity was worthy to explore. It remains controversial that whether letrozole is superior to CC as an ovulation induction regimen in IUI cycles. Letrozole was used for ovulation induction in recent years, as an aromatase inhibitor, it can suppress estrogen production and reduces estrogen negative feedback on FSH secretion, then increases endogenous gonadotropin secretion without depleting estrogen receptor, therefore, letrozole may have no side effect on endometrium and is gradually used for inducing ovulation in IUI treatment[17]. One randomized controlled trial suggested the clinical pregnancy rate was significantly higher in letrozole group compared with CC group in IUI with unexplained infertility, and the phenomenon also existed in some study with PCOS females[6,16]. But in some studies, there was no significant difference between CC and letrozole regarding endometrial thickness on the day of hCG administration[18,19], rates of pregnancy, abortion and
multiple pregnancy\cite{8,20}. In our present study, there were no significant differences among letrozole, CC and hMG groups in terms of rates of clinical pregnancy, miscarriage and multiple pregnancy. The dominant follicles number of CC group was a little more than two other groups while the endometrial thickness was less, so maybe the benefit of more follicles can make good of lacking in endometrium, or the endometrial receptivity has not been damaged in spite of lower endometrial thickness. Because in China, letrozole was used for breast cancer mainly, and cannot be accepted by many couples for inducing ovulation, so CC may be still the first choice.

It was reported that sequential CC/hMG regimen could significantly reduce total dose of hMG used and improve pregnancy rate compared with low-dose step-up hMG regimen in IUI\cite{5,21}. Also in this study, both CC and LE could decrease the total dose (285.0 ± 172.5 IU and 292.5 ± 202.5 IU vs 502.5 ± 285.0 IU) and duration of hMG therapy compared with hMG only (4.2 ± 0.7 d and 4.9 ± 0.8 d vs 6.9 ± 2.3 d) obviously while the number of dominant follicles and endometrial thickness were almost similar. The rates of clinical pregnancy, miscarriage, multiple pregnancy were also not significantly different among the groups, while the OHSS rate was a little higher in hMG only group (3.6%). It suggested that CC and letrozole can promote endogenous gonadotrophin secretion in early follicular phase and decrease the need of exogenous hMG, which might be the reason of low OHSS rate. Therefore, hMG is more fit for combined treatment for CC or letrozole in IUI rather than used alone.

**Conclusion**

Ovarian simulation may improve pregnancy rate in IUI compared with nature cycle. Both CC and letrozole can reduce the duration and total dose of hMG. The role of CC reducing endometrial thickness can be corrected by hMG supplement, which will not decrease pregnancy rate, the results will challenge the opinion that CC might decrease endometrial receptivity.

**References**


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