Serum estradiol levels during controlled ovarian hyperstimulation influence the pregnancy outcome of in vitro fertilization in a concentration-dependent manner

Bo Sun Joo, Ph.D., a Sea Hee Park, M.S., a Byeong Min An, M.S., a Kyung Sue Kim, M.D., b Sung Eun Moon, M.D., b and Hwa Sook Moon, M.D., Ph.D. a,b

a Center for Reproductive Medicine; and b Department of Obstetrics and Gynecology, Good Moonhwa Hospital, Busan, South Korea

Objective: To determine an optimal serum E2 level on the day of hCG administration in controlled ovarian hyperstimulation (COH) during IVF-ET without compromising pregnancy outcome.

Design: Retrospective study.

Setting: Large urban medical center.

Patient(s): Data of 455 cycles of fresh IVF-ET with COH.

Intervention(s): Serum E2 levels on the day of hCG administration were categorized into five groups: group A (<1000 pg/mL), group B (1000–2000 pg/mL), group C (2000–3000 pg/mL), group D (3000–4000 pg/mL), and group E (>4000 pg/mL).

Main Outcome Measure(s): Serum E2 levels, number of oocytes retrieved, pregnancy outcomes.

Result(s): Of 455 cycles, 148 (32.5%) cycles resulted in clinical pregnancy. The implantation rate was 12.2%, and the delivery rate was 18.7%. The number of oocytes obtained increased with increasing serum E2 levels. The pregnancy rate gradually increased from group A to D as E2 levels increased but decreased in group E. In women <38 years, the IVF-ET outcomes were similar to those of total patients. However, in women ≥38 years old, pregnancy and delivery rates were higher in group C than in other groups.

Conclusion(s): These results show that serum E2 levels have a concentration-dependent effect on the pregnancy outcome, suggesting an optimal range of E2 level for achieving a successful pregnancy. This optimal range of serum E2 level in women is age dependent: 3000–4000 pg/mL for women <38 years and 2000–3000 pg/mL for women ≥38 years. (Fertil Steril 2010;93:442–6. ©2010 by American Society for Reproductive Medicine.)

Key Words: IVF-ET, controlled ovarian hyperstimulation, serum E2 levels, pregnancy outcome

Adequate endometrial preparation is essential for achieving and maintaining pregnancy. Estrogens play a key role in the regulation of uterine preparation for embryo implantation (1–3) via stimulation of endometrial proliferation (4, 5) and enhancement of uterine and endometrial perfusion (6–8). In this respect, exogenous E2 administration before ET has been used for satisfactory priming of the uterus (9, 10).

Implantation rates are 0–33.0% in natural cycles but are only 7%–9% in stimulated cycles despite a significant increase in the number of oocytes (11). Serum E2 concentrations during controlled ovarian hyperstimulation (COH) are increased by 10-fold compared with those of natural cycles (12). Some studies have suggested that high serum E2 concentrations resulting from excessive ovarian response adversely affect the outcomes of assisted reproduction (13, 14). On the contrary, other studies reported that high serum E2 concentrations do not appear to alter endometrial receptivity (15) or pregnancy outcome (16, 17).

Therefore, the objective of this study was to evaluate the effect of serum E2 levels on the day of hCG administration on the pregnancy outcome of IVF-ET after COH and to establish an optimal range of serum E2 concentrations without compromising the outcomes.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Good Moonhwa Hospital.

Patient Characteristics

Data from 494 cycles of IVF-ET treatment from January 2003 to December 2006 were reviewed. Excluding 39 cycles performed with donated oocytes or cases lacking serum E2 concentrations, data were obtained from 455 cycles. Serum E2 levels on the day of hCG administration were categorized into five groups: group A (<1000 pg/mL), group B (1000–2000 pg/mL), group C (2000–3000 pg/mL), group D (3000–4000 pg/mL), and group E (>4000 pg/mL).
(2000–3000pg/mL), group D (3000–4000pg/mL), and group E (>4000 pg/mL). Data on the number of oocytes retrieved, pregnancy rate, and implantation rate were analyzed. Serum E2 concentrations were measured by a commercially available chemi-immunoassay kit (Immunlite 2500; Siemens Healthcare Diagnostics Inc., Deerfield, CT).

### Controlled Hyperstimulation Induction

COH was performed by the standardized long or short protocol, the choice of which was based on patient characteristics or response during previous cycles. In the case of the long protocol, GnRH agonist (Lucrin; Abbott, France) was given daily at a dosage of 1.0 mg until ovarian suppression was observed by serum E2 and FSH levels on the third day of the menstrual cycle. Then GnRH agonist was given daily at a dose of 0.5 mg and IM injections of 1 or 2 ampoules of 75 IU of hMG (IVF-M; LG Inc., Iksan, Korea) were given in the evening depending on the follicular development. Highly purified FSH (Follimon; LG Inc., Iksan, Korea) were given in the evening depending on the follicular development. Highly purified FSH was only administered when the leading follicle became 14 mm in diameter. In the case of the short protocol, GnRH agonist was initiated at a dose of 1 mg on the first day of the menstrual cycle. Gonadotropins were administered in the same manner as in the long protocol.

Follicular development was assessed by transvaginal ultrasound, and hCG was administered when more than two leading follicles reached ≥18 mm in diameter.

### IVF-ET

Transvaginal ultrasound-guided oocyte retrieval was performed 36 hours after hCG injection. Oocyte maturation was evaluated by confirming the presence of the first polar body and radical expansion of cumulus cells from the oocyte-cumulus complex under phase-contrast microscope. Oocytes were cultured in modified HTF (mHTF) media containing 10% of human follicular fluid (FF) without glucose and inseminated with motile sperm prepared by the three-layer percoll gradient method. Fertilization was defined as the oocytes with two pronuclei 16–20 hours after insemination. Embryos were transferred to fresh mHTF media containing 20% FF and cocultured with cumulus cells in HTF media with glucose containing 20% FF on the following day. Embryos were transferred 72 hours after oocyte retrieval and classified by blastomere equalization and cytoplasmic fragment.

Clinical pregnancies were confirmed by the presence of gestational sac and fetal heart beat on transvaginal ultrasound 7 weeks after ET; implantation rate was defined as rate of gestational sac per embryos transferred, and delivery rate was defined as delivered pregnancy per cycle.

### Statistical Analysis

Results are expressed as mean ± SD. Statistical analysis was performed using unpaired Student’s t-test, χ²-test, and one-way analysis of variance. P<.05 was considered statistically significant.

### RESULTS

The mean age was 34.0 ± 4.5 years (range, 23–48), the mean serum E2 concentration on the day of hCG administration was 3745.3 pg/mL, and the number of oocytes obtained was 10.1. Of 455 cycles, clinical pregnancy was achieved in 148 cycles (32.5%). The implantation and delivery rates were 12.2% and 18.7%, respectively (Table 1). Table 2 shows the IVF-ET outcome according to serum E2 concentration on the day of hCG administration. The number of oocytes obtained increased with increasing serum E2 level, but patient age showed a significant decrease (P<.05). Pregnancy rate increased with increasing levels of serum E2 from group A to group D, reaching 50% in group D, but dropped in group E. The implantation and delivery rates were significantly increased as well, and the difference between groups was not statistically significant.

The fecundity of women decreased rapidly over 38 years old. So we investigated the effect of serum concentrations of E2 on IVF outcomes according to age, younger than 38 years old versus 38 years or older. In women <38 years old, unlike the result from total patients, there was no statistically significant difference in age according to the serum E2 level. However, the number of retrieved oocytes and clinical pregnancy rate correlated with serum E2 concentration. The number of retrieved oocytes was highest in group E. Pregnancy rate showed a gradual increase from group A to D in a serum E2 level-dependent manner but decreased in group E (30.8%). The implantation and delivery rates were significantly increased in group D compared with groups B or C (Table 3).

In patients ≥38 years old, the IVF outcome had a different pattern than that of total patients. The numbers of retrieved oocytes were significantly lower in group A compared with groups B, C, D, and E. Group E has a significantly higher number of oocytes retrieved than groups A, B, and C. Group C had a significantly higher pregnancy and implantation rate compared with other groups. Groups C and D had similar delivery rates (22.2% and 12.5%, respectively), which were significantly higher than other groups (Table 4).

### DISCUSSION

COH for IVF-ET is essential in improving the pregnancy rate, but supraphysiologic levels of E2 are inevitably attained during COH. The effect of such supraphysiologic E2 levels on the outcome of IVF-ET have remained controversial. Some investigators have reported that serum E2 concentrations on the day of hCG administration have a positive correlation with the pregnancy outcome. However, others reported a detrimental influence of high E2 levels on the IVF outcome or no association between the serum E2 levels and IVF outcome (18, 19).

The present study shows that the pregnancy and implantation rates increased gradually as serum E2 levels increased up to 4000 pg/mL but began to fall in concentrations above
4000 pg/mL. This result can also be observed from the study of Blazar et al., who reported that ongoing pregnancy rates were increased steadily with increasing levels of peak serum E2, until a plateau was reached at approximately 2500 pg/mL (20). The difference in serum E2 level showing the highest pregnancy rate between our study and Blazar et al.’s may be due to the difference in the method of hormonal analysis. However, both studies suggest that there is an optimal range of serum E2 level affecting the outcome of IVF-ET and that the maximum number of oocytes retrieved does not always correlate with higher pregnancy rate. In previous studies, Van der Gaast et al. suggested that milder ovarian stimulation is needed to produce fewer but higher quality oocytes without compromising endometrial receptivity and implantation (21).

Mitwally et al. grouped the area under the curve for E2 levels (AUC-E2) from the first day of COH until the day of hCG administration into the low, average, and high AUC-E2 groups and compared the pregnancy rates among the three groups (22). They showed significantly higher pregnancy rates in the average AUC-E2 group compared with low and high AUC-E2 groups. There was a positive correlation between AUC-E2 and pregnancy rates up to a certain AUC-E2 level above which a negative correlation was found. It seems that optimizing serum E2 levels on hCG administration may help improve the treatment outcome after IVF-ET but that low or high E2 levels have deleterious or no effects. This result confirms that proper estrogenization of the uterus is necessary for uterine preparation to embryo implantation (22).

Another interesting finding of this study is that the effect of serum E2 level on the day of hCG administration on the number of retrieved oocytes and the pregnancy rate depends on women’s age. In women ≥38 years, the pregnancy rate was highest with serum E2 levels of 3000–4000 pg/mL and the mean number of oocytes retrieved was 12.7. On the other hand, in women <38 years old, it was highest with serum E2 levels of 2000–3000 pg/mL, although the mean number of oocytes was 5.0. This supports the result of Mitwally et al., who showed that supraphysiologic levels of E2 have a detrimental influence on IVF outcome in patients 35 years or older. That is, older women seem to be more vulnerable to the deleterious effect of high E2 levels than younger women (22). In the present study, such a poor outcome was obvious in women ≥38 years old, and the effect of serum E2

### TABLE 1
**Patient characteristics and IVF outcomes.**

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>455</td>
</tr>
<tr>
<td>Age, years</td>
<td>34.0 ± 4.5</td>
</tr>
<tr>
<td>E2 on hCG treatment</td>
<td>3745.3 ± 2902.9 pg/mL</td>
</tr>
<tr>
<td>No. of retrieved oocytes</td>
<td>10.1 ± 7.0</td>
</tr>
<tr>
<td>No. of transferred embryos</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>148 (32.5)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>183 (12.2)</td>
</tr>
<tr>
<td>Delivery rate (%)</td>
<td>85 (18.7)</td>
</tr>
</tbody>
</table>

**Note:** Values are mean ± SD unless otherwise indicated.


### TABLE 2
**Comparison of IVF outcome according to the serum E2 levels on the day of hCG administration.**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>72</td>
<td>100</td>
<td>66</td>
<td>50</td>
<td>167</td>
</tr>
<tr>
<td>Age, years</td>
<td>36.8 ± 5.3</td>
<td>35.3 ± 4.2</td>
<td>33.3 ± 4.1a,b</td>
<td>33.1 ± 4.1a</td>
<td>32.7 ± 3.8a,b</td>
</tr>
<tr>
<td>No. of retrieved oocytes</td>
<td>3.1 ± 2.6</td>
<td>5.9 ± 3.2a</td>
<td>8.2 ± 4.7a,b</td>
<td>12.0 ± 6.0a,b,c</td>
<td>15.3 ± 6.6a,b,c,d</td>
</tr>
<tr>
<td>No. of ETs</td>
<td>2.2 ± 1.1</td>
<td>3.1 ± 1.2a</td>
<td>3.5 ± 1.2a</td>
<td>3.7 ± 0.9a</td>
<td>3.6 ± 1.0a</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>16 (22.2)</td>
<td>32 (32.0)</td>
<td>25 (37.8)</td>
<td>25 (50.0)a,b</td>
<td>50 (29.9)d</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>21 (13.0)</td>
<td>35 (11.2)</td>
<td>27 (11.5)</td>
<td>35 (19.0)</td>
<td>66 (10.8)</td>
</tr>
<tr>
<td>Delivery rate (%)</td>
<td>10 (13.9)</td>
<td>13 (13.0)</td>
<td>13 (19.7)</td>
<td>16 (32.0)a,b</td>
<td>33 (19.8)</td>
</tr>
</tbody>
</table>

**Note:** Values are mean ± SD unless otherwise indicated.

a P < .05 (vs. group A).
b P < .05 (vs. group B).
c P < .05 (vs. group C).
d P < .05 (vs. group D).

concentration on the outcome of IVF-ET was dose and women’s age dependent. However, our study has a limitation in coming to the conclusion that women 38 years had the highest delivery rates in group C compared with other groups due to the small number of one or two deliveries. Therefore, further study is needed to confirm that conclusion for large cases.

Estrogen is a major angiogenic and vasodilative factor resulting in improving endometrial proliferation and uterine perfusion for the priming of embryo implantation (6, 7, 25). Owing to this property, it is expected that estrogen contributes to successful pregnancy.

The negative effect of serum E2 on the day of hCG may be explained by two mechanisms. First is the cellular and tissue change of oocytes and endometrium exposed to E2 for a long time and damage of endometrial receptivity due to a change in the ratio of E2 to P (26). Second, increasing levels of E2 are deleterious to embryonic implantation because they directly affect the embryo (23). However, the role of receptor of GnRH on uterine endometrium in COH cycles with GnRH agonist or antagonist was not clearly elucidated, so it is difficult to confirm that the change of endometrium results from the change of estrogen concentration. On the contrary, Di-Luigi and Nulsen suggested that impairment of corpus luteum function in COH for IVF-ET was due to mechanically

### TABLE 3

Comparison of IVF outcome according to the E2 levels on the day of hCG administration in women <38 years old.

<table>
<thead>
<tr>
<th>&lt;38 Years</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>42</td>
<td>73</td>
<td>57</td>
<td>42</td>
<td>147</td>
</tr>
<tr>
<td>Age, years</td>
<td>33.0 ± 2.7</td>
<td>33.3 ± 2.7</td>
<td>32.1 ± 2.9</td>
<td>31.9 ± 3.2</td>
<td>31.8 ± 2.9</td>
</tr>
<tr>
<td>No. of retrieved oocytes</td>
<td>4.0 ± 2.9</td>
<td>5.8 ± 3.0</td>
<td>8.7 ± 4.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>12.7 ± 6.2&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>15.8 ± 6.6&lt;sup&gt;a,b,c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of ETs</td>
<td>2.4 ± 1.2</td>
<td>3.2 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>13 (31.0)</td>
<td>27 (37.0)</td>
<td>22 (38.6)</td>
<td>24 (57.1)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>48 (32.7)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>18 (17.3)</td>
<td>29 (12.5)</td>
<td>24 (11.9)</td>
<td>34 (22.5)</td>
<td>64 (11.9)</td>
</tr>
<tr>
<td>Delivery rate (%)</td>
<td>9 (21.4)</td>
<td>12 (16.4)</td>
<td>11 (19.3)</td>
<td>15 (35.7)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>32 (21.8)</td>
</tr>
</tbody>
</table>

Note: Values are mean ± SD unless otherwise indicated.

<sup>a</sup>P<.05 (vs. group A).
<sup>b</sup>P<.05 (vs. group B).
<sup>c</sup>P<.05 (vs. group C).
<sup>d</sup>P<.05 (vs. group D).


### TABLE 4

Comparison of IVF outcome according to the E2 levels on the day of hCG administration in women ≥38 years old.

<table>
<thead>
<tr>
<th>≥38 Years</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>30</td>
<td>27</td>
<td>9</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Age, years</td>
<td>41.9 ± 3.2</td>
<td>40.7 ± 2.2</td>
<td>40.6 ± 3.1</td>
<td>39.8 ± 1.8</td>
<td>39.9 ± 1.9</td>
</tr>
<tr>
<td>No. of retrieved oocytes</td>
<td>1.9 ± 1.1</td>
<td>6.0 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.1 ± 5.1&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of ETs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 1.0</td>
<td>3.0 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>3 (10.0)</td>
<td>5 (18.5)</td>
<td>3 (33.3)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1 (12.5)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 (10.0)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>3 (5.2)</td>
<td>6 (7.3)</td>
<td>3 (9.8)</td>
<td>1 (3.0)</td>
<td>2 (2.7)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Delivery rate (%)</td>
<td>1 (3.3)</td>
<td>1 (3.7)</td>
<td>2 (22.2)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1 (12.5)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1 (5.0)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Values are mean ± SD unless otherwise indicated.

<sup>a</sup>P<.05 (vs. group A).
<sup>b</sup>P<.05 (vs. group B).
<sup>c</sup>P<.05 (vs. group C).

disrupted corpus luteum by the process of follicular aspiration for oocyte retrieval rather than by elevated E2 concentrations as granulosa cells are simultaneously aspirated (27).

In conclusion, we found that serum E2 levels during COH influence the IVF outcome in a concentration-dependent manner. This result suggests that there is an optimum range of serum E2 levels that positively affect IVF outcome. The optimal range of E2 levels is age dependent. In women ≥ 38 years old, elevated serum E2 levels were more detrimental to implantation compared with in younger women. Our results suggest 3000–4000 pg/mL for women < 38 years and 2000–3000 pg/mL for women ≥ 38 years as an optimal range of E2 levels. Therefore, taking the pregnancy rate and ovarian hyperstimulation syndrome into consideration, COH for IVF-ET should aim at an optimum rather than maximum number of oocytes without compromising uterine receptivity or embryo implantation.

REFERENCES


