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

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Objective: To determine an optimal serum E_2 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 E_2 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 E_2 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 E_2 levels. The pregnancy rate gradually increased from group A to D as E_2 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 E_2 levels have a concentration-dependent effect on the pregnancy outcome, suggesting an optimal range of E_2 level for achieving a successful pregnancy. This optimal range of serum E_2 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 E_2 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 E_2 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 E_2 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 E_2 concentrations resulting from excessive ovarian response adversely affect the outcomes of assisted reproduction (13, 14). On the contrary, other studies reported that high serum E_2 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 E_2 levels on the day of hCG administration on the pregnancy outcome of IVF-ET after COH and to establish an optimal range of serum E_2 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 E_2 concentrations, data were obtained from 455 cycles. Serum E_2 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

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(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 E_2 concentrations were measured by a commercially available chemiimmunoassay 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 E_2 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)/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 $\geq$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 $\pm$ SD. Statistical analysis was performed using unpaired Student's *t*-test, χ^2-test, and one-way analysis of variance. $P<.05$ was considered statistically significant.

RESULTS

The mean age was 34.0 $\pm$ 4.5 years (range, 23–48), the mean serum E_2 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 E_2 concentration on the day of hCG administration. The number of oocytes obtained increased with increasing serum E_2 level, but patient age showed a significant decrease ($P<.05$). Pregnancy rate increased with increasing levels of serum E_2 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 E_2 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 E_2 level. However, the number of retrieved oocytes and clinical pregnancy rate correlated with serum E_2 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 E_2 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 $\geq$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 E_2 are inevitably attained during COH. The effect of such supraphysiologic E_2 levels on the outcome of IVF-ET have remained controversial. Some investigators have reported that serum E_2 concentrations on the day of hCG administration have a positive correlation with the pregnancy outcome. However, others reported a detrimental influence of high E_2 levels on the IVF outcome or no association between the serum E_2 levels and IVF outcome (18, 19).

The present study shows that the pregnancy and implantation rates increased gradually as serum E_2 levels increased up to 4000 pg/mL but began to fall in concentrations above