Objective: To examine the relationships between peak serum estradiol (E2) levels and treatment outcome in in vitro fertilization (IVF) cycles after embryo transfer (ET) on day 3 or day 5.


Setting: A university-affiliated assisted reproduction program.

Patient(s): Infertile patients undergoing IVF-ET cycles.

Intervention(s): Peak E2 concentration in serum was determined on the day of human chorionic gonadotropin (hCG) administration. The IVF-generated embryos were cultured for 2 days until transfer on day 3. If more than four 8-cell embryos were present on day 3, embryo culture was continued until day 5 for blastocyst transfer.

Main Outcome Measure(s): Clinical pregnancy rates.

Result(s): High peak E2 levels did not adversely affect treatment outcome. After the cycles were divided according to the day of ET, high peak E2 levels were associated with improved pregnancy rates after ET on day 5 but not on day 3.

Conclusion(s): Increasing peak E2 levels in IVF cycles are associated with improved pregnancy rates after ET on day 5. (Fertil Steril 2003;80:75–9. ©2003 by American Society for Reproductive Medicine.)

Key Words: Estradiol level in serum, blastocyst transfer, pregnancy rates

The primary aim of controlled ovarian hyperstimulation (COH) in in vitro fertilization-embryo transfer (IVF-ET) cycles is to produce a large cohort of mature oocytes for IVF. Elevated secretion of ovarian steroid hormones is inevitably associated with COH. Serum estradiol (E2) levels can be increased more than 10-fold over those found during spontaneous cycles (1, 2). Because cyclic changes in the endometrium are regulated by ovarian steroid hormones, the increased ovarian steroid hormone secretion from COH may compromise endometrial receptivity for embryo implantation (3–5). However, conflicting reports exist concerning the impact of COH on pregnancy rates after IVF-ET (6–8).

In vivo in humans, the embryo arrives in the uterus 4 to 5 days after natural fertilization. Studies on mice and rats have demonstrated that the uterine environment is hostile to embryos if they are placed in the uterus prematurely (9). Traditionally embryos are transferred to the uterus on day 2 or 3 after IVF. Recent advances in embryo culture technology have permitted the delay of the transfer to day 5, when the embryos have reached the blastocyst stage in vitro (10–12). Transfer of blastocysts follows the physiologic “schedule” of embryo arrival in the uterus, when the uterine environment is probably the most supportive for continued embryo development and implantation. Moreover, the uterine environment may be more receptive because the 2- to 3-day delay of transfer may afford the uterus more time to recover after being exposed to the peak
of the superphysiologic levels of E₂. This retrospective study
determined the effect of high E₂ levels on pregnancy rates
after ET on day 3 or day 5.

MATERIALS AND METHODS

This study included 697 IVF-ET cycles, representing one
cycle from each patient (only the first cycle was included for
patients who repeated treatments) between January 1999 and
December 2001 at the Northwestern University Medical
School. Cycles that used donor eggs or frozen-thawed em-
byros were excluded from this study. This study was exempt
from institutional review board review because it involved
retrospective chart reviews but no patient intervention or
specific contact, and it did not disclose any patient identity
information.

Controlled ovarian hyperstimulation was achieved by
once or twice daily SC injections of gonadotropins after
pituitary down-regulation with an agonist of gonadotropin-
releasing hormone (GnRH-a). Typically, patients received
0.5 mg GnRHa (Lupron, TAP Pharmaceutical, North Chi-
cago, IL) daily for 21 days to suppress endogenous gonad-
otropic secretion. Once the absence of dominant follicular
development was confirmed sonographically and serum E₂
levels were reduced to less than 30 pg/mL, FSH administra-
tion was initiated at daily dosages ranging from 150 to 450
IU. Final follicular maturation was triggered by the admin-
istration of 10,000 IU human chorionic gonadotropin (hCG)
when at least two follicles were more than 18 mm in diam-
eter. Transvaginal oocyte retrieval was performed 36 hours
after hCG administration.

Oocytes were inseminated 4 to 6 hours after retrieval by
coculture with motile sperm or by intracytoplasmic sperm
injection (ICSI), depending on semen quality. Fertilization
was assessed 15 to 18 hours after insemination. Embryos
were cultured under mineral oil in 40 μL droplets of culture
medium at 37°C in a humidified, 5% O₂, 5% CO₂, and 90%
N atmosphere until day 3. Transfer was performed on day 3,
or delayed until day 5 if more than four 8-cell embryos were
present on day 3. Sequential media from SAGE Biopharma
(Bedminster, NJ) or Irvine Scientific (Santa Ana, CA) were
used for embryo culture. Luteal support was provided by IM
injections of P in oil at a dosage of 50 mg twice daily,
beginning immediately after oocyte retrieval. To assess treat-
ment outcome, serum beta hCG was measured 14 days after
retrieval in all patients and repeated 2 days later if the first
result was positive. Clinical pregnancy was confirmed by
ultrasound observation of fetal cardiac activity 5 to 6 weeks
after retrieval. Implantation rates were determined by divid-
ing the total number of sonographically observed fetal hearts
with the total number of embryos transferred.

Stepwise logistic regression analysis using SPSS software
(SPSS Inc, Chicago, IL) assessed the dependence of treat-
ment outcome (clinical pregnancy) on peak E₂ levels, patient
age, number of days of gonadotropin stimulation, number of
large follicles with a diameter greater than 15 mm on the day
of hCG administration, and number of mature oocytes re-
trieved. The analysis was carried out separately for day 3 and
day 5 transfers. The cycles were then divided according to
peak E₂ levels (high: top 25th percentile; medium: medium
50th percentile; and low: lower 25th percentile) and the day
of ET (day 3 or day 5). These groups were compared with
Student’s t-test or chi-square test.

RESULTS

During the period between January 1999 and December
2001, 134 cycles were canceled due to poor ovarian response
to gonadotropin stimulation (i.e., the number of mature fol-
cicles was fewer than three and serum E₂ level was less than
500 pg/mL by day 10 of stimulation). None of the cycles that
appeared to lead to hyperstimulation was cancelled before
ovocyte retrieval. However, during the study period, 14 oo-
cyte retrievals were not followed by transfer of the fresh
embryos due to ovarian hyperstimulation. All of these 14
patients returned for transfers of frozen-thawed embryos,
resulting in five clinical pregnancies. The average patient age
for the 697 complete IVF-ET cycles included in this study
was 35.3 years. A clinical pregnancy rate of 45% was
achieved from these cycles. The dependence of treatment
outcome (clinical pregnancy) on patient age and three vari-
able related to ovarian stimulation was determined by lo-
gistic regression analysis (Table 1). A statistically significant
association was found for treatment outcome with both pa-
tient age and peak E₂ level. When analysis was performed
separately according to the day of transfer, a statistically
significant association was found for treatment outcome with
peak E₂ levels only in the day 5 transfer group, and with
patient age only in the day 3 transfer group.

The distribution of major diagnoses among the low, me-
dium, and high peak E₂ level groups is presented in Figure 1.
Patients with polycystic ovarian syndrome were most likely
to have very high peak E₂ levels (P<.05, chi-square test).
None of the causes of infertility appeared to predispose
patients to having a low peak E₂ level.

Comparison between Different Peak E₂ Levels
and the Day of Transfer

In the high peak E₂ level group, the patients were
younger, had a greater number of oocytes available for
retrieval, and had fewer embryos transferred in comparison
with the low peak E₂ level group (Table 2). Both implanta-
tion and pregnancy rates were higher in the high peak E₂
level group than in the medium and low peak E₂ level
groups. When separated by the day of transfer, implantation
and pregnancy rates were significantly higher in the high
peak E₂ level group than in the low peak E₂ level group only
in the day 5 transfer cycles. In general, the guidelines of the
American Society for Reproductive Medicine were followed
with regard to the number of embryos transferred as a
function of patient age. Most day-5 transfers consisted of no more than two embryos. In 18 transfers, three embryos were replaced owing to the patient’s age (older than 38 years) and poor blastocyst quality; one of these transfers resulted in a triplet birth.

**DISCUSSION**

High E2 levels resulting from ovarian hyperstimulation have been shown to reduce pregnancy rates in rats and mice because of the adverse effects on the reproductive tract and embryo development (13–16). Several studies of human IVF-ET cycles have also suggested that high peak E2 levels negatively influence treatment outcome (6, 7). However, this adverse effect has not been verified in other clinical studies. Anderson-Sykes et al. (17) and Ng et al. (18) found that the developmental potential of embryos produced from IVF cycles with high E2 levels was not compromised in subsequent thaw-transfer cycles. Young IVF patients who responded to COH with high E2 levels had pregnancy rates that were similar to patients who received donor eggs without being subjected to COH (8).

Gidey-Baird et al. (19) suggested that an altered ratio between E2 and P levels, rather than E2 levels alone, may mediate the negative impact of COH on treatment outcome. Premature luteinization has also been implicated in mediating the potentially negative effect of COH (20, 21). Because we did not monitor P levels in our patients, we cannot evaluate any possible interaction between P and E2. In our study, high peak E2 levels did not adversely affect pregnancy rates. On the contrary, high peak E2 levels were associated with improved treatment outcome for day-5 transfers. High E2 levels in response to COH probably reflect a healthy

**TABLE 1**

Logistic regression analyses of possible effects of age and gonadotropin-induced ovarian responses on treatment outcome.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Patient age</th>
<th>Peak E2 level</th>
<th>Days of stimulation</th>
<th>No. of large follicles</th>
<th>No. of mature eggs retrieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>-0.047</td>
<td>-0.026</td>
<td>&lt;0.001</td>
<td>0.076</td>
<td>-0.026</td>
</tr>
<tr>
<td>P</td>
<td>.050</td>
<td>.567</td>
<td>.303</td>
<td>.001</td>
<td>.503</td>
</tr>
</tbody>
</table>

Note: R = regression coefficient. R is considered statistically significant if P<.05.

**FIGURE 1**

Distribution of diagnoses among low (upper 25%, or <1289 pg/mL), medium (medium 50%, or 1289–2495 pg/mL), and high (lower 25%, or >2495 pg/mL) peak E2 levels. More patients with polycystic ovaries were in high peak E2 levels than patients with other diagnoses (P<.05, chi-square test).

ovarian reserve. The patients who had higher E2 levels were younger than those with lower E2 levels and, therefore, were more likely to produce oocytes with greater developmental potential (22).

However, the benefits associated with high E2 levels may be offset by the negative impact of COH on the endometrium or other target tissues, particularly if the embryos are placed in the uterus soon after the E2 level peak for oocyte retrieval. Our data suggest that such a hypothetical effect on the endometrium may, to some extent, dissipate between day 3 and day 5. Accordingly, the beneficial effect of high E2 levels with respect to oocyte quality may become predominant when the embryos are transferred on day 5.

Although high E2 levels appeared to be associated with improved treatment outcome, extremely high levels were predictive of severe ovarian hyperstimulation syndrome, a health hazard to be avoided. During the 3-year period examined by this study, 14 cycles were not completed with ET because of severe ovarian hyperstimulation syndrome. Six of the 14 patients were hospitalized for 1 to 2 days for fluid control.

In agreement with previous studies (22–24), our study demonstrated that treatment outcome also depends on the patient’s age. However, when day-3 and day-5 transfers were analyzed separately, the impact of age was only statistically significant for day-3 transfers. The lack of randomization and the selection of transfer day based on the number of 8-cell embryos present on day 3 likely introduced selection bias, which may have diminished the effect of age on treatment outcome for the day-5 transfers.

The recent advances in embryo culture system have permitted extended culture of IVF-generated embryos to the blastocyst stage. Transfer of blastocysts can lead to improved pregnancy rates and permit a reduced number of embryos to be transferred (10–12). In our study, only one of the six triplet pregnancies was a result of a day-5 transfer among the low E2 group. The improvement in pregnancy rates with day-5 transfers is most likely a result of increased selection pressure on the embryos during the extended culture period. However, the possibility cannot be ruled out that treatment outcome may also benefit from a more synchronized, supportive uterine environment on day 5 versus day 3. Transferring embryos on day 5 may afford the uterus more time to recover from any potentially negative impact caused by the exposure to high peak E2 levels. If these data are validated in large-scale studies, it may be argued that patients with unusually high peak E2 levels may be more likely to benefit from delaying ET to day 5.

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### References


