

# Supraphysiological estradiol levels do not affect oocyte and embryo quality in oocyte donation cycles

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**BACKGROUND:** The study aim was to determine whether supraphysiological estradiol (E<sub>2</sub>) levels reduce oocyte/embryo quality in oocyte donation cycles. **METHODS:** A retrospective analysis of 330 consecutive fresh oocyte donation cycles was performed in an assisted reproductive treatment programme between January 1996 and December 2000. Throughout the study period, oocyte donors and recipients followed a standard synchronization regimen that did not vary. A serum E<sub>2</sub> level (peak E<sub>2</sub>) was obtained from all oocyte donors on the morning of HCG administration. Peak E<sub>2</sub> values were grouped by 33rd percentile (group I, <1500 pg/ml; group II, 1500–3000 pg/ml; and group III, >3000 pg/ml). All embryo transfers were performed on day 3 after oocyte recovery. **RESULTS:** Comparisons between groups revealed no significant differences in the quality of oocytes retrieved, and in fertilization rates. Higher peak E<sub>2</sub> levels were directly correlated with a greater number of oocytes retrieved, embryos available for transfer and cryopreservation, and higher average embryo quality scores ( $P < 0.005$ ). Compared with group I, group III had significantly higher embryo implantation rates ( $P < 0.05$ ). **CONCLUSIONS:** Sustained supraphysiological E<sub>2</sub> levels do not adversely affect the quality of developing oocytes and embryos. On the contrary, elevated E<sub>2</sub> levels are associated with a larger number of oocytes and embryos and high-grade embryos for transfer/cryopreservation and, consequently, improved implantation rates.

*Keywords:* embryo/estradiol/oocyte/oocyte donation

## Introduction

Assisted reproductive therapies use controlled ovarian stimulation to recruit a large cohort of oocytes in order to maximize the interaction of gametes and increase the chances for pregnancy. As a result, an exaggerated elevation of serum estradiol (E<sub>2</sub>) level occurs, followed by an alteration in the ratio of serum progesterone to E<sub>2</sub>. The physiological impact on the quality of the cycle as a result of an elevated E<sub>2</sub> remains the subject of intense debate (Sharara, 1999, 2000; Simón and Pellicer, 1999; Ng, 2000).

For lack of a natural in-vivo human model, IVF–embryo transfer cycles have been used as an alternative way to study the effects of peak E<sub>2</sub> on embryo quality and endometrial receptivity. Some investigators have noted no adverse effects, while others demonstrated evidence of significant decreases in fertilization, implantation and pregnancy rates (Forman *et al.*, 1988; Pellicer *et al.*, 1989, 1996; Chenette *et al.*, 1990; Toner *et al.*, 1991; Simón *et al.*, 1995, 1998; Sharara and McClamrock, 1999; Ng *et al.*, 2000). Adverse effects of a supraphysiological E<sub>2</sub> may include alterations in both endometrial receptivity and oocyte/embryo quality.

Unfortunately, observations related to conventional IVF–embryo transfer may lead to erroneous conclusions, as heterogeneous diagnoses are present in this population of infertile patients. Confounding variables such as the presence of hydrosalpinges and severe endometriosis make it difficult to study individual effects related to oocyte/embryo quality and endometrial receptivity. Oocyte donation, on the other hand, represents a more ideal model because the oocyte donor's gametogenesis and ovarian steroidogenesis are dissociated from the recipient's endometrial development and receptivity. To date, only one group (Simón *et al.*, 1995) has examined the effects of elevated peak E<sub>2</sub> levels in oocyte donation cycles. However, interpretation of these data was difficult because the recipients received supernumerary oocytes from high responder (defined as  $\geq 15$  retrieved oocytes) infertile patients who themselves were undergoing IVF–embryo transfer. In contrast to Simón *et al.* (1995), in the present study an oocyte donation model was chosen in which each recipient received all the oocytes retrieved from one designated donor. These donors were typically young (21–34 years of age), healthy and not infertile, and represented a more homogeneous cohort than infertile populations. Similarly, the endometrial

receptivity of the recipient was relatively constant because of uniform artificial preparation. In this way, it was possible to evaluate the consequences of peak E<sub>2</sub> on oocyte/embryo quality independent of the effects on endometrial receptivity. The purpose of this study was to define these independent factors using the oocyte donation model.

## Materials and methods

A total of 330 consecutive fresh oocyte donation cycles performed on 272 recipients using 186 different oocyte donors between January 1996 and December 2000 was retrospectively reviewed. The protocol for oocyte donation was reviewed and approved by the institutional review board of the College of Physicians & Surgeons, Columbia University. Oocyte donors were aged <35 years, with normal baseline day 3 FSH levels (i.e. <15 mIU/ml). Patients who had undergone embryo transfer on days 2 or 5 after retrieval, who had testicular sperm aspiration or semen from donor plus partner, or with 'shared' oocyte donor cycles were excluded from the analysis. Thus, in a study cycle, a single recipient received all the oocytes from a designated donor. No donor was an infertile women undergoing assisted reproduction.

The characteristics of the recipients were as follows [mean ± SEM (range)]: age 43.5 ± 0.3 (26–56) years; gravidity 1.42 ± 0.13 (0–10); parity 0.40 ± 0.06 (0–4); male factor 50.6%. The characteristics of the donors were: age 25.9 ± 0.2 (20–34) years; gravidity 0.64 ± 0.06 (0–6); parity 0.30 ± 0.04 (0–4).

Oocyte donors and recipients followed a standard synchronization regimen that was previously described (Sauer *et al.*, 1989, 1992) and did not vary throughout the study period. Briefly, all donors underwent ovarian down-regulation using leuprolide acetate. Once down-regulation was confirmed, leuprolide acetate was decreased and gonadotrophin (FSH or HMG) was started at a dose of two to four ampoules per day. The ovarian response was assessed by ultrasound and serum E<sub>2</sub> level on the fifth day of gonadotrophin, and serially thereafter. HCG (10 000 IU) was administered intramuscularly when lead follicles reached 19–20 mm in diameter. A serum E<sub>2</sub> (peak E<sub>2</sub>) level was obtained from all oocyte donors on the morning of HCG administration. Ultrasound-guided transvaginal oocyte retrieval was performed 34–36 h later under intravenous conscious sedation. All visible follicles, regardless of diameter, were aspirated from both ovaries. At the time of retrieval, all recovered oocytes were graded for maturity (grade 2 oocyte with a light fluffy cloudy appearance and a radiating corona radiata). Oocytes were either inseminated or injected (ICSI) depending on sperm quality. Semen criteria for ICSI in the study included the following: sperm concentration <20 × 10<sup>6</sup>/ml or <50% motility or <4% normal forms by Kruger strict criteria. Only embryos with two visualized pronuclei (2PN) were considered normally fertilized.

Recipients were synchronized using oral micronized E<sub>2</sub> approximately 5 days before the donor-initiated gonadotrophin therapy. Recipients with gonadal function (*n* = 155; 57%) underwent leuprolide acetate down-regulation before starting E<sub>2</sub>. Progesterone was started on the morning of the day before the donor's retrieval and continued twice daily thereafter. All embryo transfers were performed 72 h post-retrieval. Before embryo transfer, embryos were graded according to a scale of 1 to 5 based on modified criteria established previously (Veeck, 1991). A grade 5 embryo would be optimal (embryo stage appropriate for time with equal size blastomeres, clear cytoplasm and no cytoplasmic fragments), while grade 1 embryo would be very poor quality (embryo stage not appropriate for time, irregular blastomeres with poor quality cytoplasm and significant fragmentation). To

assess overall quality of the transferred embryo, an embryo score was calculated by multiplying the rating of the embryo by the number of its blastomeres. The scores of all embryos transferred per patient were summed and then divided by the number of embryos transferred to give the average embryo score (AES). Excess embryos of high-quality (grade 4–5) were then cryopreserved using a programmable freezer.

Following embryo transfer (*n* = 330), patients continued with supplemental E<sub>2</sub> and progesterone until a negative pregnancy test was documented 12 days later or when pregnant until 14 weeks of estimated gestational age. Clinical pregnancy was defined as the presence of a gestational sac on ultrasound. Ongoing pregnancy was defined as a continuing pregnancy after 20 weeks gestation. Abortions were defined as losses prior to 20 weeks gestation. Peak E<sub>2</sub> levels were grouped by 33rd percentile (group I <1500 pg/ml; group II 1500–3000 pg/ml; and group III >3000 pg/ml).

In order to examine the effects of E<sub>2</sub> on the developmental capacity of oocytes and embryos, the following outcome measures were compared: number of oocytes retrieved; ratio of grade 2 oocytes to total oocytes retrieved; fertilization rates; number of embryos cryopreserved; number of embryos transferred; and cumulative embryo score. In addition, clinical outcomes were also studied including clinical pregnancy, ongoing/delivered pregnancy rate per embryo transfer and implantation rates.

Serum levels of E<sub>2</sub> were measured by chemiluminescent enzyme immunoassays (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). Intra-assay and inter-assay coefficients of variation did not exceed 9.3 and 10.5% respectively. Statistical analyses were performed using SPSS-PC (SPSS Inc., Chicago, IL, USA). Statistical differences between groups were determined by analysis of variance (ANOVA), *t*-test and  $\chi^2$  test. Correlations were analysed using the Pearson product moment correlation. All data were expressed as mean ± SEM unless otherwise noted. A *P*-value < 0.05 was considered significant.

## Results

The overall clinical pregnancy rate per transfer was 42.7% and the embryo implantation rate was 20.7% with an ongoing/delivered pregnancy rate per transfer of 37.2%.

Comparisons between groups revealed no differences in recipient age, or in the proportion of male factor patients that required ICSI (Table I). With regard to donor profile, significant differences in age, gravidity and parity (*P* < 0.05) were noted between groups, although there were no differences in baseline day 3 FSH and E<sub>2</sub> levels. Patients in group III required the lowest number of ampoules of gonadotrophin for ovarian stimulation, but had the highest number of oocytes retrieved, number of 2PN, number of embryos transferred and cryopreserved (Table I). Pearson correlations revealed that donors with higher peak E<sub>2</sub> levels used fewer ampoules of gonadotrophin (*r* = -0.21, *P* < 0.005) and required fewer days of stimulation (*r* = -0.18, *P* < 0.005). There were no significant differences in oocyte quality, since the fertilization rate and the ratio of grade 2 oocytes to total oocytes were unchanged (Table I). On the other hand, the AES of embryos transferred was significantly higher in groups II and III than in group I (*P* < 0.05) (Table I). A direct correlation was noted between peak E<sub>2</sub> level and AES (*r* = 0.213, *P* < 0.005). The number of embryos cryopreserved was also significantly higher in

**Table I.** Oocyte donation cycle characteristics by group<sup>a</sup>

Characteristic	Group I	Group II	Group III
No. of cycles	79	141	110
Recipient age (years)	42.9 ± 0.6	43.6 ± 0.4	44.0 ± 0.5
Male factor requiring ICSI (%)	42.9	51.1	47.1
Donor age (years)	27.0 ± 0.4	25.7 ± 0.3*	25.4 ± 0.4*
Donor gravidity	1.38 ± 0.29	1.43 ± 0.17*	1.47 ± 0.23*
Donor parity	0.25 ± 0.07	0.49 ± 0.10	0.43 ± 0.13*
Donor day 3 FSH (mIU/ml)	5.6 ± 0.5	4.7 ± 0.4	4.3 ± 0.2
Donor day 3 E <sub>2</sub> (pg/ml)	32.3 ± 2.6	36.5 ± 2.8	37.2 ± 2.2
Total ampoules of gonadotrophins	34.4 ± 1.0	30.6 ± 0.6*	29.8 ± 0.7*
E <sub>2</sub> after 4 days of FSH (pg/ml)	95.3 ± 8.3	169.1 ± 10.1**	354.7 ± 22.5**†
Peak E <sub>2</sub>	1050 ± 35	2188 ± 38**	4216 ± 109**†
Oocytes retrieved	10.0 ± 0.5	16.2 ± 0.5**	26.2 ± 0.8**†
Group 2 oocytes:total oocytes ratio	0.55 ± 0.02	0.53 ± 0.02	0.54 ± 0.02
Fertilization rate (%)	60	59	59
2PN embryos (n)	5.9 ± 0.3	9.5 ± 0.4**	15.4 ± 0.6**†
Embryos transferred (n)	3.8 ± 0.1	4.2 ± 0.1**	4.2 ± 0.1**
Average embryo score (AES)	27.1 ± 1.2	30.2 ± 0.8**	31.5 ± 0.8**
Embryos cryopreserved (n)	1.1 ± 0.2	3.3 ± 0.3**	6.4 ± 0.5**†

<sup>a</sup>Peak E<sub>2</sub> levels: group I = <1500 pg/ml; group II = 1500–3000 pg/ml; group III = >3000 pg/ml.

\**P* < 0.05 versus group I, \*\**P* < 0.005 versus group I.

†*P* < 0.005 versus group II.

Values are mean ± SEM.

**Table II.** Pregnancy outcome of recipients by group<sup>a</sup>

	Group I	Group II	Group III
Implantation rate (%)	17.4	19.3	24.5*
Chemical pregnancy per ET (%)	2.8	5.9	3.7
Clinical pregnancy rate per ET (%)	38.0	41.2	47.7
Spontaneous abortion rate per ET (%)	4.2	5.8	2.7
Ongoing/delivered rate per ET (%)	33.8	34.6	44.0
Singletons (%)	69.6	48.9	48.9
Twins (%)	21.7	31.9	34.0
Triplets (%)	8.7	14.9	14.9
Quadruplets (%)	0	4.3	2.1

<sup>a</sup>Peak E<sub>2</sub> levels: group I = <1500 pg/ml; group II = 1500–3000 pg/ml; group III = >3000 pg/ml.

\**P* < 0.05 versus group I.

ET = embryo transfer.

group II than group I, with group III numbers significantly higher than both of the other groups.

Pregnancy outcome of the recipients is shown in Table II. The embryo implantation rate was significantly higher in group III than in group I (*P* < 0.05), but not group II (*P* = 0.053). Other measures of pregnancy outcome, including the clinical pregnancy rate per embryo transfer, multiple pregnancy rates and ongoing/delivered pregnancies per embryo transfer, appeared to be higher with increasing peak E<sub>2</sub>, without a concomitant rise in the rate of spontaneous abortion or chemical pregnancy (Table II). However, none of these measures reached statistical significance. Further analyses of conception cycles versus non-conception cycles revealed that patients with a clinical pregnancy had significantly more embryos transferred with higher AES (*P* < 0.05). Within these groups there were no significant differences in the rate of male factor requiring ICSI or in the donor characteristics, including peak E<sub>2</sub>.

Stepwise regression analysis showed the single factor that

most significantly predicted the donor's peak E<sub>2</sub> to be age ( $\beta = -118.4$ , *P* < 0.05) after controlling for possible confounding variables such as donor's gravidity and parity, and baseline day 3 FSH and E<sub>2</sub> levels. In a stepwise logistic regression model, the single factor selected as a significant predictor of ongoing/delivered pregnancy was the AES ( $\beta = 0.05$ , *P* < 0.05) after controlling for possible confounders such as recipient age, donor age, peak E<sub>2</sub> level, number of oocytes retrieved, ratio of grade 2 to total oocytes retrieved, number of 2PN, number of embryos cryopreserved, number of embryos transferred, and ICSI.

## Discussion

Successful implantation depends on the synchronized development of both embryos and endometrium. The general assumption is that the natural ovulatory cycle produces the ideal hormonal level for gametogenesis and endometrial receptivity (Kolb and Paulson, 1997). It is further assumed that of all the substances produced by the ovaries, E<sub>2</sub> and progesterone are most important for endometrial priming. Deviations from normal values observed in the menstrual cycle are believed to be detrimental to the quality of the developing endometrium. Specifically, excessively high E<sub>2</sub> levels observed with ovarian stimulation are thought to adversely affect embryo quality and/or endometrial receptivity.

As stated earlier, data from previous studies using IVF-embryo transfer as a model to examine the effects of high peak E<sub>2</sub> on endometrial receptivity are difficult to interpret. Although evidence of an adverse effect on endometrial morphology exists, the clinical influence of high steroid concentrations remains largely unknown. When reviewing studies in which endometrial biopsies were performed during the 'implantation window' of ovarian stimulation cycles, abnormal dating was demonstrated in as many as 73% of specimens (Cittadini

and Palermo, 1991). Benadiva and Metzger found a higher incidence of dyssynchrony between endometrial glands and stroma following ovarian stimulation when compared with natural cycles (Benadiva and Metzger, 1994). Similarly, others have reported impaired development of endometrial glands (Bonhoff *et al.*, 1990), advanced endometrial histological maturity (Garcia *et al.*, 1984), stromal development (Noci *et al.*, 1997) and an earlier expression of pinopodes (Martel *et al.*, 1987; Kolb *et al.*, 1997) following ovarian stimulation. In contrast, others (Hadi *et al.*, 1994; Macrow *et al.*, 1994) failed to find any detectable morphological changes in the endometrium. However, the former of these reports noted a significant reduction in nuclear receptors for progesterone and estrogen in both glands and stroma after ovarian stimulation. These endometrial responses to ovarian stimulation may be variable, but any change in endometrial morphology and thus the window of receptivity may adversely affect implantation. The composite effects of these hormonal alterations on endometrial receptivity require further exploration.

Oocyte and embryo quality may also be influenced by serum E<sub>2</sub> secretion (Jones *et al.*, 1983; Dor *et al.*, 1986). Previous studies have reported decreased fertilization rates with higher peak E<sub>2</sub> levels (Pellicer *et al.*, 1989; Gelety and Buyalos, 1995). More indirect evidence reveals that peak E<sub>2</sub> levels in the fresh IVF–embryo transfer cycles are not related to the success of frozen–thawed embryo transfer cycles (Toner *et al.*, 1991; Schalkoff *et al.*, 1993; Ng *et al.*, 2000). Embryo quality appeared unaffected, as excess embryos from different groups of peak E<sub>2</sub> levels had similar implantation and pregnancy rates during the freeze–thaw embryo transfer cycles. Nonetheless, a strong bias may have been present since only patients who did not conceive in the fresh cycle subsequently underwent a frozen embryo transfer cycle.

Oocyte donation is a more appropriate model to study the different compartments. The recipient's endometrial receptivity is dissociated from folliculogenesis since it is artificially prepared to be more uniform and similar to that of a natural menstrual cycle. Using a mouse embryo donation model, a reduced implantation rate was demonstrated in animals which had higher E<sub>2</sub> levels following ovarian stimulation (Fossum *et al.*, 1989). Data on human oocyte/embryo donation are generally lacking, however. In one report of data from oocyte donation (Simón *et al.*, 1995), the effects of high peak E<sub>2</sub> levels in 108 cycles of 96 high-responder oocyte donations did not result in significant differences in fertilization, implantation and pregnancy rates. However, as stated previously, infertile women undergoing IVF–embryo transfer who donate their supernumerary oocytes may present with confounding variables. Although not reported, these patients may have other diagnoses that might affect their oocyte and subsequent embryo quality, and their donated oocytes may be of poorer quality because the patient herself would first receive the oocytes of higher grade. Nevertheless, these authors concluded that oocyte and embryo quality was not affected by high levels of E<sub>2</sub>. Using the model described herein, in which each recipient received all the oocytes from one single designated donor who was healthy and not infertile, such biases were avoided. In accordance with a previous report (Simón *et al.*, 1995), the

present study found that the high peak E<sub>2</sub> levels were not associated with a lower ratio of high-grade oocytes, fertilization rates or embryo quality. These data suggest that the developmental quality of oocytes and embryos obtained was not impaired by elevated E<sub>2</sub>. On the contrary, higher levels correlated directly with a greater number of oocytes retrieved, more embryos available for transfer and cryopreservation, and higher AES. Furthermore, in contrast to the previous findings (Simón *et al.*, 1995), in the present study the highest embryo implantation rate was found in the group with highest peak E<sub>2</sub> (group III).

In spite of the greater number of oocytes and embryos in groups II and III, the clinical outcomes were not significantly improved compared with group I, that is, rates of clinical pregnancy, spontaneous abortion and ongoing/delivered pregnancy per embryo transfer. Groups II and III appeared to have an increased rate of multiple pregnancies. While these differences did not reach statistical significance, the transfer of fewer embryos should be considered in these patient groups. Power calculations revealed that, with respect to clinical pregnancy rate and ongoing/delivered rate per embryo transfer, this study had a  $\beta$  value of 0.72, and 384 patients in each group would be required in order to avoid a type II error ( $\beta = 0.2$ ). Therefore, although an increase in implantation rates was observed, no conclusions could be drawn at this time regarding the effects of high peak E<sub>2</sub> levels on pregnancy outcome.

In conclusion, we consider that high peak E<sub>2</sub> levels are not detrimental to oocyte quality, fertilization and embryo cleavage. These observations also suggest that sustained supraphysiological levels of E<sub>2</sub> resulting from ovarian stimulation produce no adverse effects on the quality of developing human oocytes and embryos during IVF–embryo transfer cycles. On the contrary, elevated levels result in higher implantation rates and a greater number of oocytes and embryos for selection at the time of embryo transfer, as well as for cryopreservation. These findings imply that the possible decrease in embryo implantation rates seen with conventional IVF (Paulson *et al.*, 1990) is largely due to an endometrial effect.

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