The Influence of Supraphysiologic Estradiol Levels on Human Nidation[1]

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Objective: *Exogenous estradiol (E_2) has a well-recognized interceptive action when administered shortly after ovulation. The influence of extremely elevated levels of endogenous E_2 on human oocyte fertilization and implantation are unclear. The purpose of this study was to evaluate a potential antinidatory role of extremely high endogenous E_2 concentrations on implantation and pregnancy during* in vitro *fertilization–embryo transfer (IVF-ET).*

Methods: *Twenty-five patients receiving human menopausal gonadotropins (hMG) following midluteal GnRHa administration for IVF-ET, in which the maximal E_2 concentration was >5000 pg/ml (range 5358–16,344 pg/ml) were studied. Cycle parameters including oocyte and embryo characteristics, fertilization, cleavage, and implantation rates as well as pregnancy outcomes were compared to those of 25 patients treated contemporaneously whose treatment cycles had peak E_2 values <3500 pg/ml. Patients groups were matched for age, infertility diagnoses, duration of infertility and stimulation protocol.*

Results: *Cycles characterized by very high endogenous E_2 levels resulted in significantly more oocytes per retrieval (21.4 ± 1.7 versus 8.4 ± 0.6; $P < 0.0001$), fewer postmature oocytes (1.6% ± 1.0% versus 14% ± 5.0%; $P < 0.03$), and a decreased fertilization rate (63% ± 4.0% versus 73% ± 3.0%; $P < 0.04$) compared to control cycles. There were no differences in the overall mean morphologic grade or cleavage rates between groups. However, high E_2 cycles were associated with a significantly increased implantation rate (14% ± 4.0% versus 8.0% ± 4.0%; $P < 0.01$) and pregnancy rate per embryo transfer (62% ± 16% versus 36% ± 16%; $P < 0.01$) compared to controls. The incidence of spontaneous abortion did not differ between groups.*

Conclusions: *Extremely high endogenous E_2 levels do not appear to adversely affect implantation or overall cycle pregnancy rates in IVF-ET cycles. However, impaired fertilization rates in such cycles support a potential adverse effect on oocyte quality.*

KEY WORDS: *in vitro* fertilization; estradiol; fertilization rates; pregnancy rates.

INTRODUCTION

The peak estradiol (E_2) level achieved during controlled ovarian hyperstimulation (COH), patient age, infertility diagnosis, number of oocytes recovered and embryos transferred are all factors known to influence pregnancy outcomes in IVF-ET (1). Early studies suggested an antinidatory role for very high levels of endogenous E_2 achieved during COH (2,3). This adverse effect on implantation was attributed to the well-recognized interceptive action of exogenous E_2 administered shortly after ovulation (4). In contrast, two more recent studies have reported no adverse effect on outcomes in IVF-ET cycles associated with high endogenous E_2 levels (5,6).

It has been postulated that the high E_2 levels achieved during COH may exert adverse effects on oocyte quality and/or endometrial receptivity. However, an overall improvement in pregnancy rates in IVF-ET cycles characterized by extremely high E_2 levels may occur due to the increased number of oocytes recovered, resulting in more embryos available for transfer or cryopreservation (3). The purpose of this study was to examine the specific effect of high endogenous E_2 levels on oocyte quality, im-

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plantation rates, and cycle outcomes in a subgroup of patients achieving extremely high endogenous E_2 levels during COH for IVF-ET.

MATERIALS AND METHODS

Subjects

All subjects in this study underwent COH for the purpose of IVF-ET at the UCLA Medical Center Fertility Unit between January 1989 and January 1992. Twenty-five consecutive patients undergoing stimulation cycles characterized by peak serum E_2 levels >5000 pg/ml (range 5358–16,344 pg/ml) were analyzed. Subjects ranged in age from 27 to 36 years with a mean age of 32.2 ± 0.6 years. The principal infertility diagnoses at the time of COH for IVF-ET included: tubal factor (n = 16), polycystic ovarian syndrome (PCOS) (which was defined as chronic anovulation with associated hyperandrogenism) (n = 5), minimal endometriosis/unexplained infertility (n = 3), and male factor (n = 1). The duration of infertility ranged from 2.2 to 7.2 years with a mean of 3.7 ± 0.3 years. Twenty-five patients who were treated contemporaneously whose cycle peak serum E_2 was less than 3500 pg/ml served as controls. Control patients were matched for chronological age, infertility diagnosis, and duration of infertility and had undergone an identical ovarian stimulation protocol. Patient and cycle characteristics are shown in Table I.

Stimulation Protocol

The COH regimen used has been previously described in detail (7). Briefly, GnRHa (Lupron, TAP Pharmaceuticals, North Chicago, IL) 1.0 mg SC q.d. was begun on cycle day 21 following spontaneous or induced withdrawal bleeding and/or 7 days after detection of an urinary LH surge. On day 31, transvaginal sonography (TVS) was performed and serum E_2 assayed to ensure ovarian suppression (no ovarian cyst >10 mm diameter and serum E_2 <30 pg/ml). The GnRHa dose was reduced to 0.5 mg/d SC and hMG (Pergonal, Serono Laboratories, Randolph, MA) 225 IU/d was administered intramuscularly (IM) for 5 days. Follicular development was evaluated after 5 days by TVS and measurement of peripheral serum E_2 levels by rapid radioimmunoassay (RIA). The hMG dosage was then individualized based on follicular response which was monitored at 1- to 3-day intervals. Human chorionic gonadotropin (hCG, Profasi; Serono Laboratories) 10,000 IU IM was administered when two or more follicles with a mean diameter ≥18 mm and a serum E_2 ≥500 pg/ml was achieved. Ultrasound guided transvaginal ovum retrieval was performed 34–35 h

Table I. Selected Patient Characteristics by Group[a]

		Cases (n = 25)	Controls (n = 25)
Age	Mean	32.2 ± .7 yr	32.2 ± .7 yr
	Range	(27–36 yr)	(27–36 yr)
Diagnosis	Tubal	16 (64%)	16 (64%)
	Min/mild Endometriosis/Unexplained	3 (12%)	3 (12%)
	PCOS	5 (20%)	5 (20%)
	Male Factor	1 (4%)	1 (4%)
Duration of infertility	Mean	3.7 ± .3 yr	3.9 ± .3 yr
	Range	(2.2–7.2 yr)	(2.0–7.5 yr)
Ampules hMG	Mean	38.0 ± 1.9	41.8 ± 2.8
	Range	(26–63)	(27–76)
Cycle day HCG	Mean	10.6 ± 0.5	10.0 ± 0.4
	Range	(8–20)	(7–16)
Peak E_2 (pg/ml)	Mean	7,736 ± 536	2,096 ± 135
	Range	(5,258–16,344)	(919–3,488)
Day of peak E_2	Day of HCG	0%	0%
	Day post HCG	100%	100%
Cycles resulting in OHSS (%)		16%	4%
	mild	4% (pk E_2 8,340 pg/ml)	4%
	moderate	8% (pk E_2 8,470 & 10,965)	0%
	severe	4% (pk E_2 5,658 pg/ml)	0%

[a] Data are presented as n, mean ± standard deviation, or %.

later. Oocytes morphologic grading was classified as previously described (8). Insemination was performed 4 to 6 h after oocyte retrieval. Fertilization was assessed as previously described (9). Intrauterine transfer of 3 to 5 pronucleate through 8-cell stage embryos was performed 48 to 50 h following oocyte retrieval. Excess embryos were cryopreserved. Informed consent was obtained regarding the risks of severe ovarian hyperstimulation in all patients electing to proceed with embryo transfer with a peak serum E_2 >3500 pg/ml and/or with sonographic evidence of multiple ovarian follicles 11–15 mm. Progesterone in oil, 25 mg, was administered IM daily beginning on the day of embryo transfer (ET). Serum hCG levels were measured by RIA 14 days following ET.

The fertilization rate was defined as the number of embryos demonstrating morphologic evidence of 2 pronuclei per the number of oocytes inseminated. The cleavage rate was defined as the number of embryos cleaved (2- to 8-cell stage) at the time of intrauterine transfer per the number oocytes fertilized. The implantation rate was calculated as the number of gestational sacs observed by TVS per the number of embryos transferred. Pregnancy was defined as a rising serum hCG level and evidence of a gestational sac by TVS. Spontaneous abortion (SAB) was a defined as a pregnancy loss prior to 20 weeks gestation.

Estradiol Assay

The E_2 RIA (Pantex, Santa Monica, CA) was performed by a single laboratory using a commercially available kit. The sensitivity, intraassay and interassay coefficients of variation (CV) of the assay were 10 pg/ml, 3.0% to 4.3%, and 7.5% to 11.1%, respectively.

Statistical Methods

Statistical comparison between groups was performed using Kruskal–Wallis tests. Comparison of proportions were performed with chi-square and McNemar's modified chi-square tests for paired groups where appropriate. Differences were considered significant at $P < 0.05$.

RESULTS

Cycle characteristics among the 25 cases characterized by high E_2 levels (>5000 pg/ml) were compared with 25 control cycles matched for stimulation protocol, patient age, infertility diagnosis, and duration of infertility, and are shown in Table I. There were no differences observed between groups with respect to either the quantity or duration of hMG administration. Three of the 25 patients (16%) achieving high E_2 levels developed ovarian hyperstimulation syndrome (OHSS). However, the grade of OHSS (mild, moderate, severe) did not correlate with the peak E_2 level. An additional three patients (16%) of cycles elected cryopreservation of all embryos following aspiration to minimize the likelihood of developing severe OHSS.

The number of oocytes retrieved, oocyte morphologic grade, fertilization and cleavage rates in cycles characterized by high E_2 levels and control cycles are shown in Fig. 1. Cycles characterized by high E_2 levels had significantly more oocytes obtained per retrieval (21.4 ± 1.7 vs 8.4 ± 0.6, $P < 0.0001$) and a lower percentage of postmature oocytes (1.6% ± 1.0% vs 14% ± 5.0%, $P < 0.03$) than control cycles. A lower rate of fertilization was observed in high E_2 cycles (63% ± 4.0% vs 73% ± 3.0%, $P < 0.04$) as compared to control cycles. However, there was no difference in the mean oocyte morphologic grade (2.6 ± 0.05 vs 2.6 ± 0.06, $P = 0.45$) between groups. In addition, the cleavage rates (75% ± 4.0% vs 75% ± 4.0%, $P = 0.96$) did not differ between high E_2 and control cycles.

Additional cycle outcomes per embryo transfer in high E_2 cycles as compared with matched controls are shown in Fig. 2. No significant differences were observed between groups in either the number of embryos transferred (4.6 ± 0.2 vs 4.2 ± 0.2, $P = 0.08$) or the number of blastomeres per embryo transferred (4.3 ± 0.4 vs 4.3 ± 0.3, $P = 0.70$). However, cycles characterized by a high E_2 level were associated with a significantly increased implantation rate (14% ± 4.0% vs 8.0% ± 4.0%, $P < 0.01$) and implantation rate per number of blastomeres transferred (0.04 ± 0.01 vs 0.02 ± 0.09, $P < 0.01$). The pregnancy rate per ET was higher in the high E_2 group compared to controls (62% ± 16% vs 36% ± 16%, $P < 0.01$). There was no significant difference in the spontaneous abortion rate observed between groups (9.0% ± 6.0% vs 0%, $P = 0.16$).

DISCUSSION

It is clear that cycle outcomes in IVF-ET are influenced by several variables including patient age,

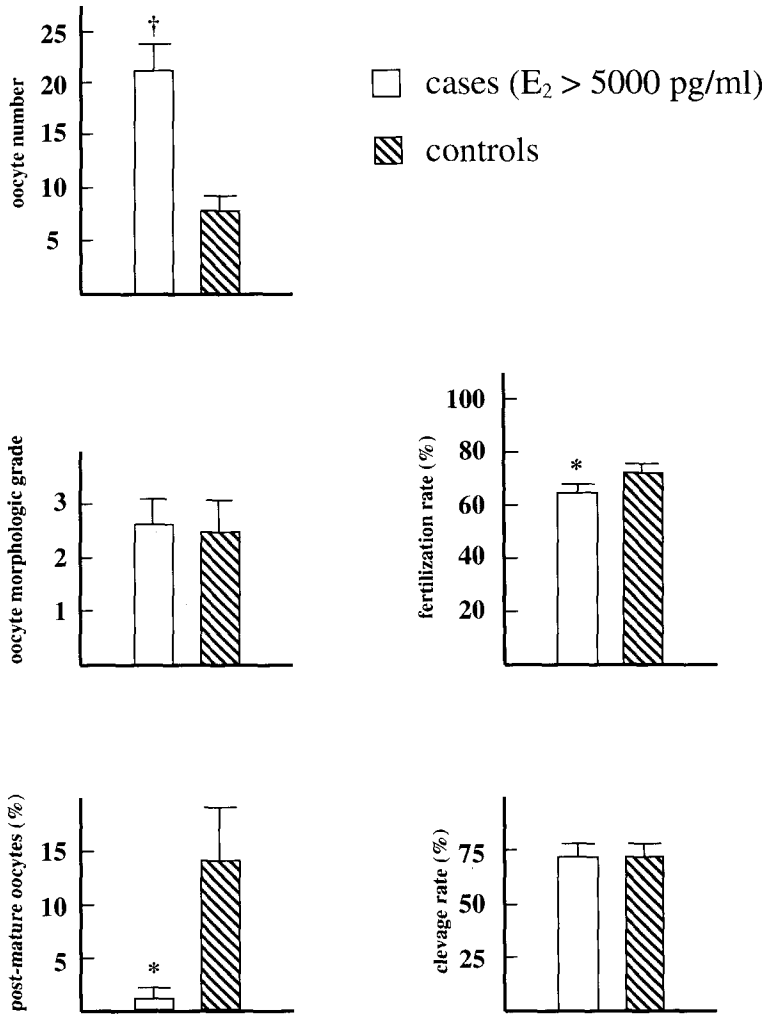

Fig. 1. Oocyte number, morphology, fertilization and cleavage rates in controlled ovarian hyperstimulation cycles characterized by high E_2 levels (>5000 pg/ml) compared with controls matched for chronological age, infertility diagnoses, duration of infertility, and stimulation protocol. * represents $P < 0.05$ and **$P < 0.001$, compared to control cycles.

infertility diagnosis, duration of infertility, and the ovarian stimulation regimen employed (1). However, the influence of extremely high endogenous E_2 levels is unclear (2,3,5,6). In order to clarify the specific effects of high levels of endogenous E_2 achieved during COH, oocyte characteristics, embryo parameters and pregnancy outcomes were compared between cycles with extremely high peak E_2 concentrations (>5000 pg/ml) to cycles with maximal values <3500 pg/ml. Furthermore, other variables known to influence cycle outcomes including patient age, infertility diagnosis, and stimulation protocol were matched between groups.

Peripheral serum E_2 levels during COH reflect the sum production by developing follicles (5). Cycles demonstrating extremely high levels of E_2 yielded significantly more oocytes at retrieval compared to age-matched controls undergoing the identical stimulation regimen. Furthermore, the amount and duration of hMG administration did not differ significantly between case and control cycles. These observations support the concept of an exaggerated ovarian response to COH in individual patients (10,11). Such a response has been observed more frequently in younger patients, those with low body mass, or experiencing chronic anovulation attributable to PCOS (11,12). However, these criteria are not entirely predictive, as demonstrated by the het-

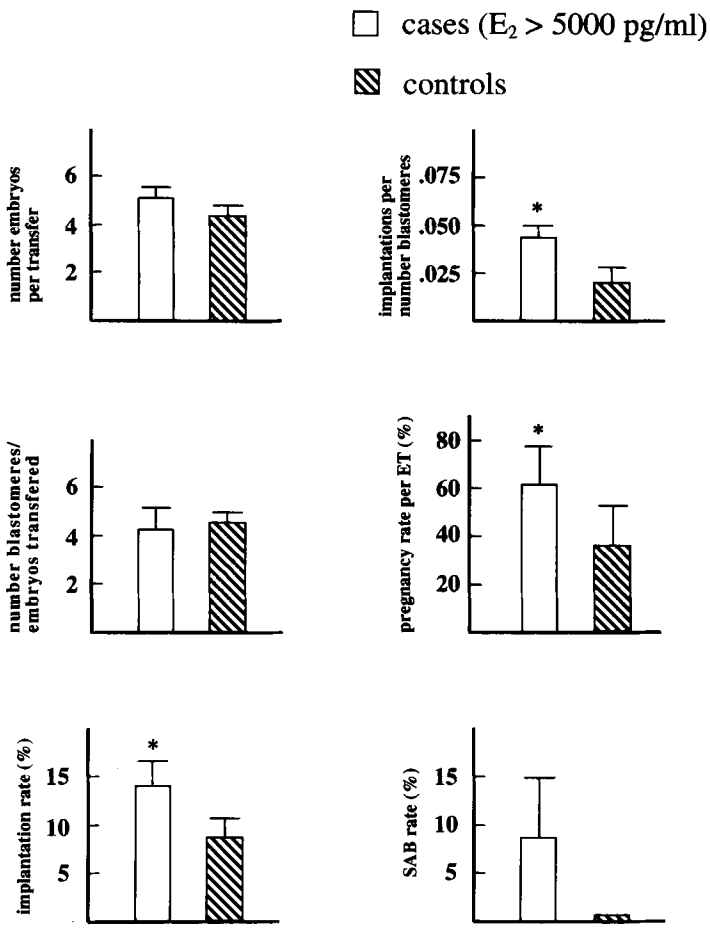

Fig. 2. A comparison of the number of embryos transferred, number of blastomeres per ET, implantation rate, implantation rate per number of blastomeres, pregnancy rates per ET, and spontaneous abortion rates in controlled ovarian hyperstimulation cycles with high E_2 levels (>5000 pg/ml) and controls cycles. * denotes $P < 0.01$, compared to control cycles.

erogeneity of the patient characteristics observed in the high-E_2 group.

There were no differences observed in the morphologic grade of oocytes in either group. When compared to cycles matched for age and infertility diagnosis, significantly fewer postmature oocytes were obtained from cycles demonstrating extremely high E_2 levels. However, the number of oocytes demonstrating evidence of cytoplasmic immaturity was not increased in the high-responder group. In addition, the duration of the follicular phase did not differ between groups. Collectively, this suggests greater synchrony among the developing follicular cohort in these brisk-responder patients, with fewer oocytes demonstrating morphologic evidence of postmaturity.

Several reports have observed a decrease in fertilization rates following exaggerated follicular stimulation using GnRHa/hMG (11–14), while Chennette *et al.* (6) reported no correlation between the maximum level of E_2 and the fertilization rate. However, these analyses did not control for critical variables of patient age and infertility diagnosis, which are known to influence fertilization rates. After controlling for these confounding variables, we observed a significant decrease in the fertilization rate in cycles characterized by extremely high E_2 levels. The etiology of the reduced rate of fertilization is unclear. Tarin and Pellicer (12), using cytogenetic analysis of inseminated-unfertilized oocytes, detected no difference in the incidence of chromosomal abnormalities among high-responder patients. They observed a higher incidence of cytoplasmic immaturity as the number of oocytes recov-

ered increased and hypothesized that this may be responsible for lower fertilization rates. It is possible that subtle morphologic or functional oocyte immaturity not detected by standard morphologic evaluation, may account for the impaired fertilization observed in our high-responder group. This is supported by the observation that once fertilized, cleavage rates were similar between groups.

It has been hypothesized that the increased pregnancy rates observed with oocyte donation for IVF-ET may be attributed in part to the avoiding a potential adverse effect of supraphysiological concentrations of E_2 on the recipient endometrium (15). Similarly, elevated serum E_2 levels resulting from COH have been implicated in impaired endometrial receptivity resulting in decreased implantation and pregnancy rates following embryo transfer in IVF (2,3). A potential antinidatory role of E_2 in COH cycles is based largely on the observed interceptive action of high doses of exogenously administered estrogens given shortly after ovulation for the purpose of postcoital contraception (4). Increasing experience gained using the model of ovum donation has demonstrated that the synchronization between endometrial histologic development and implantation may be less precise than previously theorized. High implantation and pregnancy rates have been demonstrated despite wide variations in circulating E_2 levels and follicular phase length (5 to 35 days) (16). Furthermore, the postovulatory window of implantation in the human has been shown to extend over a minimum of 6 consecutive days (16). This suggests that subtle histologic abnormalities induced by fluctuations in serum E_2 levels may have minimal influence on implantation. This is congruent with our observations that neither implantation rates or pregnancy rates were adversely affected in cycles characterized by extremely high endogenous E_2 levels, after controlling for both the number of embryos and the number of blastomeres per embryo replaced. Furthermore, the rate of early pregnancy wastage was not increased in patients in the high E_2 group.

The severity of OHSS was not found to correlate with the peak E_2 levels in this study population. Although pregnancy rates are often increased, COH cycles manifesting extremely high E_2 levels are at increased risk for the development of severe ovarian hyperstimulation syndrome (17). The use of intravenous serum albumin appears to be a useful adjunct for preventing severe OHSS in patients at high risk for development of this condition (18). Additionally, cryopreservation of all embryos for future transfer is a viable option for reducing the incidence and severity of OHSS (19,20). These options may provide effective alternatives to cycle cancellation in these high-responder patients.

In summary, we found no evidence of an adverse effect of extremely high estradiol concentrations on either implantation or pregnancy rates in IVF-ET cycles. These findings do not support an antinidatory role for high endogenous E_2 levels in the transfer cycle. The decreased fertilization rate observed in COH cycles characterized by high E_2 levels suggests a potential subtle impairment in oocyte quality which may be unique and difficult to quantify using current methodologies in high-responder patients. However, pregnancy outcomes per treatment cycle are favorable in these brisk-responder patients despite the subtle adverse effects on fertilization, due to the increased number of oocytes retrieved and favorable implantation rates in these cycles.

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