ORIGINAL ARTICLE

High Serum Estradiol Levels are not Detrimental to *In Vitro* Fertilization Outcome

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SUMMARY

Objective: To evaluate the impact of high estradiol (E2) levels and a high number of retrieved oocytes on the outcome of *in vitro* fertilization (IVF) cycles.

Materials and Methods: We retrospectively reviewed 274 IVF cycles. These patients were divided into five groups according to their peak E2 levels on the human chorionic gonadotropin day: ≤ 2,000 pg/mL (130 cycles); 2,001–3,000 pg/mL (53 cycles); 3,001–4,000 pg/mL (46 cycles); 4,001–5,000 pg/mL (29 cycles); > 5,000 pg/mL (16 cycles). Fertilization, pregnancy, and implantation rates were analyzed between these groups. We also compared the outcome of IVF for high responders (> 15 retrieved oocytes) and normal responders (≤ 15 retrieved oocytes).

Results: The oocyte fertilization and embryo cleavage rates were not significantly different among these five groups. Although decrease in pregnancy and implantation rates was observed when E2 levels were > 5,000 pg/mL compared with those having lower E2 levels, there were no statistically significant differences between these five groups. In addition, similar IVF outcome was detected for those cycles with > 15 oocytes and ≤ 15 oocytes obtained.

Conclusion: High serum E2 levels and high oocyte yield are not detrimental to IVF outcome. More studies are needed to characterize the threshold E2 levels above which implantation rates are reduced. [*Taiwanese J Obstet Gynecol* 2007;46(1):54–59]

Key Words: estradiol, IVF, pregnancy rate

Introduction

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 [3,4].

The effect of such supraphysiologic E2 levels on the outcome of IVF-ET has been the subject of intense debate with conflicting evidence [5,6]. Some investigators have shown that supraphysiologic E2 levels have a detrimental influence on endometrial receptivity and IVF outcome [7–17]. However, others did not find high E2 levels to be detrimental to IVF outcome [18–25]. Adverse effects of a supraphysiologic E2 may include alterations in both endometrial receptivity and oocyte/embryo quality.

The purpose of this study was to examine the effects of high serum E2 levels on the day of human chorionic

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gonadotropin (HCG) administration on pregnancy achievement in fresh IVF-ET cycles, in which GnRH analog was used for downregulation.

Materials and Methods

We performed a retrospective analysis of patient characteristics, measures of ovarian response, and rates of implantation and pregnancy for first cycles only of all patients < 35 years of age who underwent IVF-ET. Between January 1998 and December 2002, 274 IVF cycles were studied. These patients were divided into five groups according to their peak E2 levels on the HCG day: ≤ 2,000 pg/mL (130 cycles); 2,001–3,000 pg/mL (53 cycles); 3,001–4,000 pg/mL (46 cycles); 4,001–5,000 pg/mL (29 cycles); > 5,000 pg/mL (16 cycles). We also compared the outcome of IVF for high responders (> 15 retrieved oocytes) and normal responders (≤ 15 retrieved oocytes) defined by Simon et al [13].

All patients underwent ovarian downregulation using leuprolide acetate. Once downregulation was confirmed, leuprolide acetate was decreased and gonadotropin (follicle stimulating hormone or human menopausal gonadotropin) was started at a dose of three to four ampoules per day. The ovarian response was assessed by ultrasound and serum E2 level on the 5th day of gonadotropin, and serially thereafter. HCG (10,000 IU) was administered intramuscularly when lead follicles reached 18 mm in diameter. A serum E2 (peak E2) level was obtained on the day of HCG administration. Ultrasound-guided transvaginal oocyte retrieval was performed 34–36 hours later under intravenous conscious sedation. Oocytes were inseminated by using standard protocols or intracytoplasmic sperm injection as indicated. Embryos were transferred 72 hours after follicular aspiration. Patients undergoing IVF-ET received intramuscular progesterone in oil (50 mg/day) for luteal support starting on the day of oocyte retrieval. Pregnancies were documented by measuring serum hCG 14 days after embryo transfer.

Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA). Statistical differences between groups were determined by analysis of variance (ANOVA), *t* test, and χ^2 test. All data were expressed as mean ± standard error of the mean. A *p* value < 0.05 was considered significant.

Results

Table 1 compares the IVF outcome of normal responder (≤ 15 oocytes) with high responder (> 15 oocytes) patients. As expected, there was a significant increase in the E2 levels ($p < 0.001$) on the day of HCG injection in the high responder group. Although the high responder patients were significantly younger (30.2 ± 3.6 years *vs*. 33.6 ± 4.1 years) and the number of embryos transferred was increased (4.2 ± 1.5 *vs*. 3.8 ± 1.3), pregnancy and implantation rates were similar between two groups.

Table 2 summarizes the ovarian responses, fertilization, and cleavage rates in different groups according to the serum E2 levels on the day of HCG administration. The mean number of oocytes obtained in these five groups was 6.9, 11.5, 16.2, 21.4, and 27.3, respectively, and a statistically significant difference was found among all five groups ($p < 0.001$, ANOVA). The fertilization and cleavage rates were similar for the five groups. No differences were found among the five groups with regard to the number of embryos replaced. Although at peak serum E2 greater than 5,000 pg/mL, the pregnancy rate dropped to 25.0% and the implantation

Table 1. *In vitro* fertilization outcome in high responders (> 15 retrieved oocytes) and normal responders (≤ 15 retrieved oocytes)*

	Retrieved oocytes, *n*		*p*
	≤ 15	> 15	
Cycles, *n*	185	89	
Age (yr)*	33.6 ± 4.1	30.2 ± 3.6	< 0.05
Peak E2 level (pg/mL)*	1,714 ± 875	3,947 ± 1,093	< 0.001
Oocytes, *n**	8.3 ± 3.5	21.8 ± 5.7	< 0.001
Fertilization rate (%)*	70.2 ± 14.7	69.8 ± 13.2	NS
Embryos transferred, *n**	3.8 ± 1.3	4.2 ± 1.5	NS
Pregnancy rate, *n* (%)	63/185 (34.1)	29/89 (32.6)	NS
Implantation rate, *n* (%)	82/703 (11.7)	41/365 (11.2)	NS

**Data are presented as mean ± standard deviation. NS = not significant.*

Table 2. Ovarian responses and fertilization rates by peak E2 levels*

	Peak E2 levels (pg/mL)				
	≤ 2,000	2,001–3,000	3,001–4,000	4,001–5,000	> 5,000
Cycles, *n*	130	53	46	29	16
Age (yr)	32.9 ± 4.1	33.1 ± 4.3	32.3 ± 3.5	31.4 ± 3.9	30.1 ± 3.6
Oocytes retrieved, *n*	6.9 ± 3.1	11.5 ± 3.0	16.2 ± 3.4	21.4 ± 5.2	27.3 ± 10.0
Fertilization rate (%)†	70.3 ± 18.6	69.8 ± 20.1	70.7 ± 15.9	71.1 ± 21.1	69.7 ± 15.6
Cleavage rate (%)†	89.4 ± 21.3	91.0 ± 23.5	90.3 ± 19.8	90.7 ± 27.8	91.1 ± 25.0
Embryos transferred, *n*†	3.8 ± 1.1	4.1 ± 0.9	4.0 ± 1.2	3.9 ± 1.3	4.4 ± 1.1
Pregnancy rate, *n* (%)†	46/130 (35.4)	21/53 (39.6)	14/46 (30.4)	9/29 (31.0)	4/16 (25.0)
Implantation rate, *n* (%)†	63/491 (12.8)	26/214 (12.1)	19/184 (10.3)	12/109 (11.0)	6/70 (8.6)

**Data are presented as mean ± standard deviation; †there were no statistically significant differences between the different groups.*

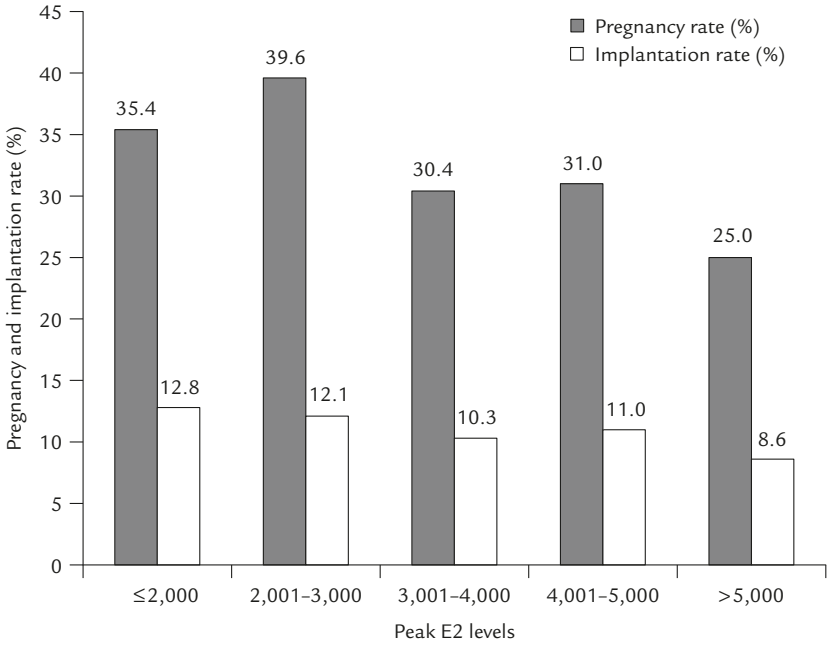

Figure. Pregnancy rates and implantation rates in groups with different E2 levels.

rate also dropped to 8.6%, there were no statistically significant differences among these five groups about the pregnancy and implantation rates (Figure).

Discussion

Since the advent of assisted reproduction, investigators have sought to elucidate factors that may limit successful embryo implantation in IVF-ET cycles. Embryo quality and endometrial receptivity were identified as important determinants of reproductive outcome in assisted reproduction. 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 [26]. Deviations from normal values observed in the menstrual cycle are believed to be detrimental to the quality of the developing endometrium. Specifically, excessively high E2 levels observed with ovarian stimulation are thought to adversely effect embryo quality and/or endometrial receptivity.

Simon et al [13] defined high responders as women who had > 15 retrieved oocytes. Fifty-nine high responders and 105 normal responders (≤ 15 oocytes) were evaluated. The fertilization rates were not different, but the pregnancy and implantation rates were markedly decreased when peak E2 levels exceeded 2,500 pg/mL, regardless of the number of retrieved oocytes [13].

The results of the current study did not show any difference in implantation and pregnancy rates between cycles with ≤ 15 oocytes and with > 15 oocytes obtained (Table 1). Similar findings were also demonstrated previously by Ng et al [17] in 1,122 women undergoing IVF treatment. This reinforced the concept of a direct adverse effect of higher E2 levels (rather than the number of oocytes retrieved) on endometrial receptivity.

The detrimental effects of very high E2 levels on implantation may result from poor embryo quality, lower endometrial receptivity, or a combination of both. In accordance with a previous report [27], the present study found that the oocyte fertilization and embryo cleavage rates did not vary among these five groups (Table 2). Moreover, Ng et al [17] showed similar implantation and pregnancy rates in subsequent frozen–thawed embryo transfer cycles in those who did not become pregnant in the fresh IVF cycles with high E2 level. These clinical data indicated that oocyte and embryo quality were not affected by the high E2 level [17,27,28].

However, a decrease in pregnancy and implantation rates was observed when E2 levels were > 5,000 pg/mL compared with those having lower E2 levels from our study (Figure). Although no statistically significant differences were detected about the pregnancy and implantation rates among the five groups, it may be due to the small sample size (n = 16) in the very high E2 levels (> 5,000 pg/mL) group. Different mechanisms have been suggested to explain the adverse effect of excessively high E2 levels and are mainly focused on possible deleterious effects on the endometrium [29]. Valbuena et al [30] have suggested that high E2 levels are detrimental to endometrial receptivity and have suggested a step-down regimen to increase endometrial receptivity in high responders [16]. Although the exact mechanisms have not yet been determined, reduced implantation in high E2 levels might be related to asynchronous endometrial development with delayed glandular maturation and advanced stromal morphology [31,32], suboptimal endometrial perfusion [33–35], and aberrant uterine expression of implantation-related genes [36]. Ma et al [36] found that the window of uterine receptivity remains open for an extended period at lower estrogen levels but rapidly closes at higher levels. The uterine refractoriness that follows the receptive state at high estrogen levels is accompanied by aberrant uterine expression of implantation-related genes (COX-2 and LIF). In a recent study, Makkar et al [37] demonstrated that a high serum E2 level had a negative effect on endometrial interleukin-11 and interleukin-6 expression. Reduced interleukin-11 and interleukin-6 expression in the peri-implantation endometrium may account for the lower implantation and pregnancy rates in the excessive responders compared with those in natural cycles and moderate responders. In addition, there are possible adverse effects directly on the embryo that could reduce the chance for blastocyst adhesion and implantation [38].

Ng et al [17] showed that a reduction in implantation and pregnancy rates in cycles with serum E2 levels ≥ 5,000 pg/mL implied that fresh embryos arising from these stimulated cycles should be cryopreserved for transfer later. The resulting pregnancy rates in frozen–thawed embryo transfer cycles would not be compromised and the risk of moderate or severe ovarian hyperstimulation syndrome (OHSS) could be further reduced [17].

A recent study [39] showed high pregnancy rates and successful prevention of severe OHSS by "coasting" of hyper-responder patients in IVF. Serum E2 levels were markedly reduced from the 1st day of withholding gonadotropin to the day of HCG administration. These data suggest that the high E2 levels may not have lasting adverse effects on uterine receptivity or the adverse effects on the endometrium may be reversible by coasting. Coasting can be considered as another option for those patients with serum E2 levels ≥ 5,000 pg/mL [40].

In conclusion, our results and those of other investigators clearly show that there is still no consensus concerning any adverse role of elevated peri-implantation E2 levels on IVF outcome. We believe, however, that there is a threshold peak E2 level above which pregnancy and implantation rates are decreased, but this threshold is likely to be 5,000 pg/mL from our results and other publications [17,21] rather than 2,500 or 3,000 pg/mL as previously reported. The impairment in implantation was likely to be related to an adverse environment in the endometrium. Further studies are needed to define the optimum E2 range associated with best IVF-ET outcome.

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