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Falling estradiol levels as a result of intentional reduction in gonadotrophin dose are not associated with poor IVF outcomes, whereas spontaneously falling estradiol levels result in low clinical pregnancy rates

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BACKGROUND: Although estradiol levels remain an integral part of monitoring in most IVF programmes, the effect of falling estradiol on IVF outcome has not been adequately quantified. The objective of this study was to evaluate the effect of falling estradiol levels prior to hCG on IVF outcome. METHODS: This was a retrospective cohort study carried out in a university-based fertility clinic. A total of 112 IVF patients in whom estradiol levels fell prior to the administration of hCG were matched for age and year of treatment with 112 control IVF patients. IVF outcomes including oocytes retrieved, fertilization rate, embryos for transfer, and pregnancy rates were compared between the groups. RESULTS: Seventy per cent of women in the falling estradiol group experienced spontaneously falling estradiol levels. Spontaneously falling estradiol was associated with fewer oocytes retrieved (median 5 versus 8, $P = 0.001$), increased rates of failed fertilization (18 versus 6%, $P = 0.018$) and lower clinical pregnancy rates (12 versus 26%, $P = 0.012$) compared to controls. Despite marked decreases in estradiol levels, IVF outcomes for patients whose estradiol levels fell as a result of deliberate protocol modification had similar fertilization and clinical pregnancy rates as controls. CONCLUSIONS: Subtle (<10%) spontaneous decreases in estradiol levels are associated with very poor IVF outcomes.

Key words: coasting/estradiol/falling estradiol/IVF outcome

Introduction

Serum estradiol (E_2) measurement remains an integral component of cycle monitoring in most IVF programmes. The pattern of follicular rise in estradiol is a useful adjunct in the identification of both poor responders, and women at risk for ovarian hyperstimulation syndrome. High basal estradiol levels (day 3), particularly in association with high basal FSH levels, predict poor ovarian response to stimulation (Licciardi *et al.*, 1995; Smotrich *et al.*, 1995). Low peak estradiol levels are often associated with advanced maternal age, reduced number of oocytes retrieved, and decreased fertilization and embryo cleavage rates (Sharma *et al.*, 1988; Phelps *et al.*, 1998). The prognostic significance of various patterns of rise of estradiol both before and after hCG administration remains unclear, and serum estradiol level alone at the time of hCG has been shown to be a poor predictor of IVF outcome. Jones *et al.* (1983) reported that increasing estradiol levels at the time of hCG administration were associated with higher success rates in IVF, whereas more poor quality oocytes, high rates of failed fertilization and fragmented embryos were associated with a plateau or decline in estradiol levels (Ben-Rafael *et al.*, 1986). These studies were all undertaken prior to the advent of the current GnRH agonist protocols.

On the other hand, women at risk of ovarian hyperstimulation maintained excellent pregnancy rates in spite of experiencing significant drops in serum estradiol prior to hCG as a result of deliberate reductions or omissions of gonadotrophin doses. The occurrence of pregnancy as well as the development of ovarian hyperstimulation syndrome (OHSS) were reported to be unrelated to trends in estradiol concentrations in a group of women 'coasted' prior to hCG (Egbase *et al.*, 2000). The objective of this study was to further characterize the effect of falling estradiol levels prior to hCG on IVF outcomes.

Materials and methods

This retrospective cohort study was carried out in the Reproductive Biology Unit of Mount Sinai Hospital, the tertiary referral centre affiliated with the University of Toronto. Mount Sinai Hospital

Institutional Research Ethics Board approval was obtained prior to commencement of the study.

Records of all IVF cycles from the period 1993–2002 were reviewed to identify women whose E_2 levels fell prior to the day of hCG administration. For patients undergoing more than one treatment cycle during the above time-period, only data from the first treatment cycle in which estradiol fell were included in the analysis. All stimulation protocols during this period involved down-regulation with GnRH agonists in either a standard long or flare protocol. Appropriate controls whose E_2 levels continued to rise until the day of hCG were selected from the same time-period and matched to cases for age and year of treatment.

Demographic information including age and diagnosis were collected for each patient. Stimulation parameters including protocol, total FSH dose, protocol modification (step-down or coasting), peak estradiol levels, and day of hCG were collected for both groups. Outcome data collected included number of oocytes retrieved, fertilization rate, number of 48–72 h embryos, number of embryos transferred, implantation rate and clinical pregnancy rate. Given that the first pregnancy ultrasound performed in our programme is at 8 weeks gestational age, the implantation rate was defined as the number of gestational sacs identified on ultrasound at 8 weeks gestational age per number of embryos transferred, and clinical pregnancies were defined as those with fetal heart activity documented on ultrasound at 8 weeks gestational age.

Women whose E_2 levels fell spontaneously and as a result of protocol modification (step-down or coasting) were analysed compared to controls to elicit the significance of this on IVF outcome.

Statistical analysis was carried out using SPSS (Version 11.0.1, SPSS Inc.). Normally distributed continuous variables were analysed using one-way analysis of variance (ANOVA), whereas non-normally distributed continuous variables were analysed using the Kruskal–Wallis one-way ANOVA. *Post hoc* analysis was performed using Dunnett's *t*-test to compare the spontaneously falling E_2 and protocol modification groups to the control group for statistical significance. Categorical variables were analysed using χ^2-test. $P < 0.05$ was considered statistically significant.

Results

One hundred and twelve patients with falling E_2 prior to hCG were matched according to age and year of treatment to 112 control patients whose E_2 levels continued to rise until the day of hCG. Thirty-four patients (30%) experienced falling E_2 as a result of intentional protocol modifications aimed at reducing the risk of ovarian hyperstimulation (FSH dose step-down or coasting). Seventy-eight patients (70%) experienced a spontaneous drop in their E_2 level prior to administration of hCG.

Patients in all three groups were similar in age and choice of treatment protocol, whereas women in the protocol modification group were more likely to have a diagnosis of anovulation compared to women in the spontaneously falling E_2 or control groups (Table I). The percentage decrease in E_2 was significantly greater in the protocol modification group than in those whose E_2 levels fell spontaneously [median 22.5% (range 1–95) versus 10% (range 1–95); $P < 0.0001$, Kruskal–Wallis test].

Despite requiring significantly higher total doses of FSH (median 2400 IU, range 225–6300) than women in the protocol modification (median 1275 IU, range 250–6375) and control groups (median 1988 IU, range 375–4800; $P < 0.0001$, Kruskal–Wallis test), women in the spontaneously falling E_2 group had lower peak E_2 levels, and lower E_2 levels on the day of hCG than the other groups (Table I). The mean (SD) cycle length expressed as the day on which hCG was given was similar between the control, protocol modification and spontaneously falling E_2 groups [day 13.4 (2.3) versus day 13.4 (1.5) versus day 14.0 (2.3) respectively, $P = 0.110$, one-way ANOVA].

The number of oocytes retrieved was greatest in the protocol modification group (median 11, range 1–34, $P = 0.005$ compared to controls, Dunnett's *t*-test) as might be expected given that a greater percentage of these women had polycystic ovarian syndrome. The spontaneously falling E_2 group, however, had fewer oocytes retrieved than the control group (median 5, range 1–27 versus median 8, range 1–34; $P = 0.002$, Dunnett's *t*-test). In addition, the spontaneously falling E_2 group experienced higher rates of failed fertilization ($P = 0.024$, χ^2-test) resulting in 24% of cycles with no embryo transfer in this

Table I. Patient demographics and cycle parameters

	Control ($n = 112$)	Protocol modification ($n = 34$)	Spontaneously falling estradiol (E_2) ($n = 78$)	*P*
Age (years), mean ± SD	34.4 ± 3.9	33.3 ± 3.5	34.9 ± 4.3	0.157[a]
Diagnosis, *n* (%)				
Tubal	78 (70)	24 (70)	59 (75)	0.005[b]
Unexplained	26 (23)	3 (9)	13 (17)	
Anovulatory	3 (3)	6 (18)	1 (1)	
Male factor	5 (4)	1 (3)	5 (7)	
Protocol, *n* (%)				
Long	84 (76)	28 (82)	57 (72)	0.500[b]
Flare	26 (24)	6 (18)	22 (28)	
Peak estradiol (pmol/l), median (range)	8134 (1022–37 446)	14 626 (3315–53 970)	5470 (298–24 144)	<0.0001[c,d]
Estradiol at hCG (pmol/l), median (range)	8134 (1022–37 446)	8046 (782–46 304)	4928 (290–19 300)	< 0.0001[c,e]

[a]One-way analysis of variance.
[b]χ^2-Test.
[c]Kruskal–Wallis test.
[d]Dunnett's *t*-test: $P = 0.001$ protocol modification versus control; $P = 0.001$ spontaneously falling E_2 versus control.
[e]Dunnett's *t*-test: $P = 0.891$ protocol modification versus control; $P < 0.0001$ spontaneously falling E_2 versus control.

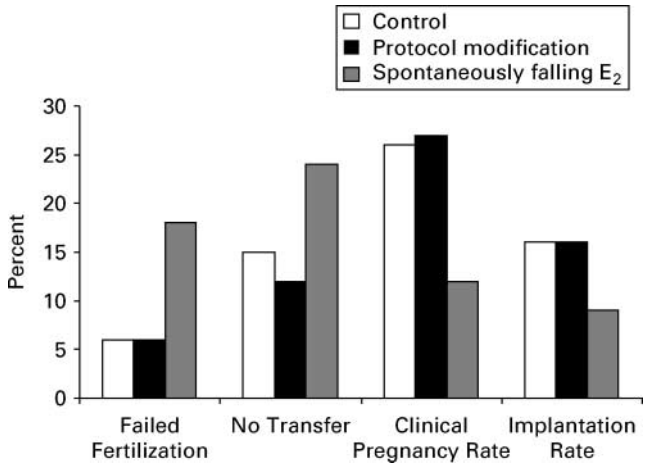

Figure 1. IVF outcomes including the rate of failed fertilization, percentage of patients with no embryos for transfer, clinical pregnancy rate and implantation rate. Significantly higher rates of failed fertilization and no embryo transfer, and lower clinical pregnancy and implantation rates were observed in the spontaneously falling estradiol group compared to the protocol modification and control groups.

group ($P = 0.07$, χ^2-test) (Figure 1). The overall fertilization rate was not significantly different between the groups (control 62%, protocol modification 66%, spontaneously falling E_2 60%; $P = 0.862$, Kruskal–Wallis test).

The mean (SD) number of healthy embryos of sufficient quality for transfer or freezing was assessed at 48–72 h and was found to be significantly different between the groups [control 4.4 (3.4), protocol modification 5 (4.0), and spontaneously falling E_2 3.1 (2.8), $P = 0.004$, one-way ANOVA]. When *post hoc* analysis was performed using Dunnett's *t*-test, there was no difference between the control and protocol modification groups ($P = 0.673$) whereas there were statistically fewer embryos at 48–72 h in the spontaneously falling E_2 group ($P = 0.015$, Dunnett's *t*-test).

Similar mean (SD) numbers of embryos were transferred in each group [control 2.6 (0.8), protocol modification 2.6 (0.7) and spontaneously falling E_2 2.4 (0.7), $P = 0.28$, one-way ANOVA]. The vast majority of embryo transfers were performed at 48–72 h, reflecting the standard practice during most of the time period of study. Two of 99 (2%) control cycles, three of 29 (10%) protocol modification cycles, and one of 60 (1.6%) spontaneously falling E_2 cycles were performed at the blastocyst stage ($P = 0.052$, χ^2-test).

Overall, there was no correlation between percentage decrease in estradiol level and clinical pregnancy rate with IVF. There were a total of 34 pregnancies in 112 cycles started (30%) in the control group (29 viable intrauterine pregnancies, three spontaneous abortions, one ectopic and one chemical pregnancy). In the protocol modification group, nine women conceived in 34 cycles started (26%), of which one pregnancy was a chemical pregnancy. Of a total of 13 pregnancies in the 78 cycles started in the falling E_2 group (17%), there were two spontaneous abortions, one ectopic pregnancy and one chemical pregnancy. Spontaneously falling E_2 was associated with a much lower clinical pregnancy rate ($P = 0.039$, χ^2-test) and implantation rate ($P = 0.244$, one-way ANOVA) than both the protocol modification and control groups (Figure 1). Of the clinical pregnancies identified, the multiple pregnancy rate was 31% (9/29) for the control group (eight twins, one triplet), 38% (3/8) for the protocol modification group (two twins, one triplet), and 33% (3/9) for the spontaneously falling E_2 group (three twins, zero triplets) ($P = 0.768$, χ^2-test). There was no statistically significant difference between the groups with respect to pregnancy outcome ($P = 0.703$, χ^2-test) or multiple pregnancy rate ($P = 0.768$, χ^2-test).

Discussion

With the high cost and invasive nature of IVF, fertility clinics continue to strive to identify prognostic factors to assist patients in making decisions regarding IVF treatment. Many static (basal estradiol, basal FSH, inhibin B, anti-Müllerian hormone, peak E_2 level, progesterone level) and dynamic (clomiphene citrate challenge test, and GnRH stimulation test) biochemical tests have been evaluated for their value in predicting IVF success. Similarly, ultrasound predictors of success including ovarian volume, antral follicle count, and appearance of healthy versus atretic pre-ovulatory follicles continue to emerge (Fukuda *et al.*, 1995).

In this study, there was no correlation between the percentage decrease in E_2 level and IVF outcome. The aetiology of the falling E_2 was a more important predictor of outcome than the magnitude of the drop in E_2. Of the 112 patients who exhibited falling E_2 levels in this study, 70% fell spontaneously, whereas the remainder fell as a result of protocol modification. Although there was no difference between the groups with respect to other important prognostic indicators such as age and choice of protocol (flare versus long protocol), women in the spontaneously falling E_2 exhibited many features of poor responders. Specifically, this group had lower peak E_2 levels, lower E_2 levels on the day of hCG and fewer oocytes retrieved despite receiving significantly higher total doses of FSH. This observation underscores the importance of continued estradiol measurement as an adjunct to ultrasound monitoring in IVF as the pattern of E_2 rise prior to hCG may provide useful prognostic information for the current and potentially future cycles.

It would seem that spontaneously falling E_2 is a reflection of the underlying health of the cohort of follicles recruited. In early studies prior to the advent of GnRH analogue use in down-regulated cycles, falling E_2 prior to hCG was presumed to be related to follicular atresia and/or premature luteinization (Jones *et al.*, 1983; Ben-Rafael *et al.*, 1986). This resulted in retrieval of greater numbers of atretic oocytes, low fertilization and cleavage rates, high rates of polyspermy and ultimately low pregnancy rates. In such cycles, it is not uncommon to witness a minor LH surge prior to follicular maturity which is sufficient to cause premature luteinization, and falling estradiol levels as androstenedione production is reduced in favour of progesterone synthesis, thus limiting the substrate for aromatization to estradiol (Erickson *et al.*, 1985).

It is clear from both animal and human studies that the fate of the developing follicle is closely related to its ability to make estradiol. *In vitro* studies have shown that estradiol has important autocrine and paracrine roles within the developing follicle. In granulosa cells, estradiol enhances FSH-stimulated cell division, induces aromatase activity, and stimulates the expression of E_2, LH and FSH receptors as well as the production of inhibin (Goldenberg *et al.*, 1973; Dorrington *et al.*, 1975; Louvet and Vaitukaitis, 1976; Erickson *et al.*, 1979; Adashi and Hsueh, 1982; Kessel *et al.*, 1985; Hillier *et al.*, 1989). Androgen production is inhibited by estradiol in surrounding theca cells (Magoffin and Erickson, 1982). Follicular fluid estradiol production has been shown to directly reflect aromatase activity *in vitro* (Hillier *et al.*, 1981), which in turn correlates with granulosa cell number and follicular maturity (size) (McNatty *et al.*, 1979). It is possible that women with spontaneously falling E_2 exhibit this pattern either due to a reduction in the absolute number of granulosa cells, or to a relative reduction in aromatase activity within the developing follicle. Both of these may be the result of a relative insensitivity to FSH which is characteristic of poor responder patients.

Regardless of the aetiology of the low estradiol levels, the effect of low estradiol levels, and particularly the E_2:androgen ratio in follicular fluid, has been well documented (Andersen, 1993; Fukuda *et al.*, 1995; Akaboshi *et al.*, 1998). It has been previously proposed that gonadotrophins and estrogens suppress the apoptotic DNA fragmentation associated with follicular atresia, whereas androgens induce follicular atresia (Billig *et al.*, 1993). Administration of diethylstilboestrol (DES) and estradiol to hypophysectomized rats resulted in prevention of granulosa cell apoptosis, whereas removal of DES and treatment with androgen were associated with increased DNA fragmentation (Billig *et al.*, 1993). Similarly, lowering androstenedione levels by active immunization has been shown to reduce follicular atresia and increase ovulation rates in sheep (Scaramuzzi *et al.*, 1980).

Clinical studies evaluating follicular fluid steroidogenesis have similarly correlated low E_2:androgen ratios with IVF outcome. Follicular fluid samples yielding an oocyte known to result in clinical pregnancy after IVF were analysed compared to follicular fluid from non-conception cycles (Andersen, 1993). Pregnancy potential of the oocytes correlated with a high E_2:androgen ratio in follicular fluid, suggesting that low E_2:androgen ratios reflect early follicular atresia and reduced viability of the associated oocyte. Similar results have been reported by others (Fukuda *et al.*, 1995; Akaboshi *et al.*, 1998).

This study was retrospective and data involving follicular fluid E_2:androgen ratios and serum androgen levels were not available. If one infers from the basic science and clinical literature that small spontaneous drops in estradiol prior to hCG reflect early follicular atresia and compromised oocyte quality, it is not surprising that this group experience high rates of failed fertilization (18%), low implantation rates (9%) and low clinical pregnancy rates per cycle started (12%). What remains to be seen is whether this is a repetitive phenomenon in subsequent IVF cycles.

In contrast to the spontaneously falling E_2 group, women whose E_2 fell as a result of intentional protocol modification aimed at reducing the risk of ovarian hyperstimulation had IVF outcomes similar to control patients. This group was more likely to have a diagnosis of 'anovulation' than either the control or spontaneously falling E_2 group, reflecting the increased risk of hyperstimulation in patients with anovulation due to polycystic ovarian syndrome. As might be expected, this group required lower doses of FSH, and achieved greater numbers of oocytes than the spontaneously falling E_2 or control groups.

Despite greater numbers of oocytes retrieved, women in the protocol modification group had similar numbers of healthy embryos on day 3 as the control group. IVF outcomes (rate of failed fertilization, fertilization rate, implantation rate, and clinical pregnancy rate) were comparable between the protocol modification and control groups.

Although it seems that falling E_2 as a result of protocol modification does not have a detrimental effect on IVF outcome, this comparison is obviously limited by lack of an appropriate control group.

Given that PCOS patients notoriously respond well to gonadotrophin stimulation, one would expect that this group might have an improved prognosis with IVF compared to control patients, many of whom have unexplained infertility. A more appropriate control group might include PCOS patients not requiring protocol modification due to concern regarding hyperstimulation.

In summary, it is clear that the aetiology of falling estradiol rather than the magnitude of the decline in estradiol is critical to IVF outcome. Patients experiencing sharp declines in estradiol level as a result of deliberate protocol modification may be reassured that this phenomenon does not compromise IVF outcomes. On the other hand, patients experiencing subtle but spontaneous declines in estradiol level should be counselled that this is associated with very poor IVF outcomes, and cycle cancellation may be warranted.

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