Does the estradiol level on the day of human chorionic gonadotropin administration predict the clinical outcome of controlled ovarian hyperstimulation?

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Summary

Objective: To investigate the effect of serum estradiol (E_2) levels on the day of human chorionic gonadotropin (hCG) administration on the outcome of controlled ovarian hyperstimulation (COH) in both long gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist protocols. *Materials and Methods:* This study included 212 in vitro fertilization-embryo transfer (IVF-ET) cycles performed with either long GnRH agonist or GnRH antagonist protocols were classified into three groups according to serum E_2 levels measured on the day of hCG injection: < 2,000 pg/ml, 2,000–4,000 pg/ml, and > 4,000 pg/ml. The three groups were compared according to age, number of retrieved oocytes, number of transferred embryos, and pregnancy rates for each of the stimulation protocols. *Results:* The long and antagonist protocols were performed in 130 and 82 cycles, respectively. The pregnancy rates were 21.5% (28/130) and 23.2% (19/82) in the long- and antagonist-protocol groups, respectively. Serum E_2 levels were measured on the day of hCG administration as < 2,000 pg/ml in 65 cycles, 2,000–4,000 pg/ml in 76 cycles, and > 4,000 pg/ml in 71 cycles. The number of retrieved oocytes increased in parallel to serum E_2 levels ($p = 0.001$). However, there was no significant difference among groups in the pregnancy rates ($p = 0.116$). Similarly, the number of retrieved oocytes increased in parallel to serum E_2 levels in both of the protocol groups (p value was 0.001 in both long GnRH agonist and antagonist protocols), but there was no correlation between the pregnancy rates and serum E_2 levels (p value of long GnRH agonist protocol was 0.254 and the p value of antagonist group was 0.349). *Conclusion:* The serum E_2 level on the day of hCG administration does not predict the pregnancy outcome in IVF with either long GnRH agonist or GnRH antagonist protocols.

Key words: Infertility; Assisted reproduction; Estradiol; In vitro fertilization; Pregnancy.

Introduction

Endometrial receptivity and embryo quality are two major factors in the implantation process. Estrogen is essential for the preparation of the endometrium for implantation, as it affects both endometrial proliferation and augmentation of uterine and endometrial perfusion. Because supraphysiologic levels of serum estradiol (E_2) are reached during controlled ovarian hyperstimulation (COH), the impact of E_2 levels on pregnancy and implantation rates has been a topic of concern.

The results of previous investigations have been controversial. Although some studies have suggested a positive correlation between serum E_2 levels and pregnancy rates [1, 2], the majority have failed to find any such relationship [3-5]. On the other hand, a decrease in the pregnancy rate due to the detrimental effects of high E_2 levels on both uterine receptivity and oocyte/embryo quality has been demonstrated [6, 7].

In most studies on this topic, gonadotropin-releasing hormone (GnRH) agonists were used for the prevention of a premature luteinizing hormone (LH) surge during gonadotropin stimulation [8-10], and GnRH antagonists were utilized in a few studies [11]. In this study, we aimed to investigate the relationship between serum E_2 levels on the day of human chorionic gonadotropin (hCG) administration and the outcome of COH in both long GnRH agonist and GnRH antagonist protocols.

Materials and Methods

We analyzed the data of 212 assisted reproductive technology (ART) cycles performed at the Reproductive Endocrinology Unit of the Istanbul Faculty of Medicine from December 2005 to December 2008. They excluded patients younger than 25 and older than 35 years, as well as the cycles in which an embryo transfer was not performed for any reason. Either a long GnRH agonist or GnRH antagonist protocol was preferred for COH based on the patient's age, ovarian reserve, and previous response to ovarian stimulation. On day 3 of the cycle, serum follicle-stimulating hormone (FSH) and E_2 levels were measured.

In the long protocol with a GnRH agonist, one mg of leuprolide acetate was administered on day 21 of the prior cycle. After a baseline ultrasonographic evaluation of the endometrium and ovaries on day 3 of the cycle, either recombinant FSH or human menopausal gonadotropin (hMG) was administered in appropriate dosages for ovarian stimulation, and the GnRH agonist dosage was reduced to 0.5 mg daily. In the GnRH antagonist protocol, ovarian stimulation was begun on day 3 of the cycle, and 0.25 mg of either cetrorelix or ganirelix was applied when the leading follicle reached 13 to 14 mm in diameter. Ovarian follicular growth was monitored daily by transvaginal ultra-

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Table 1. — *Comparisons of patient characteristics according to stimulation protocol.*

Variables	All cycles (n = 212)	Stimulation protocols Long (n = 130)	Antagonist (n = 82)	*p*
Age (years)	29.76 ± 3.14	29.51 ± 3.25	30.17 ± 2.93	0.135
Infertility duration (years)	6.62 ± 3.96	6.21 ± 3.96	7.27 ± 3.88	*0.039*
Infertility etiology (%)				*0.001*
Male	45.3	54.6	30.5	
Tuboperitoneal	23.1	18.5	30.5	
Ovarian	2.8	0	7.3	
Unexplained	28.8	26.9	31.7	
Total dose of gonadotropin (IU)	2,951.28 ± 1250.22	2,924.01 ± 1052.53	2,994.51 ± 1517.97	0.658
Day of hCG injection	12.52 ± 1.54	12.58 ± 1.43	12.41 ± 1.69	0.129
Endometrial thickness (mm)	9.86 ± 1.88	9.87 ± 2.06	9.84 ± 1.55	0.703
Serum E_2 (pg/dl)	3,272 ± 1,948.66	3,599.02 ± 2,087.07	2,753.55 ± 1,584.57	*0.001*
Retrieved oocyte number	14.51 ± 7.14	15.21 ± 7.13	13.40 ± 7.06	*0.039*
Embryo/transfer	2.87 ± 0.43	2.89 ± 0.39	2. 84 ± 0.48	0.411
Pregnancy rate (%)	22.2	21.5	23.2	0.781

Note: Values are means ± SD

Table 2. — *Comparisons of variables between the groups according to serum E_2 levels on the day of hCG administration in all cycles.*

E_2 levels	< 2,000 pg/ml (n = 65)	2,000–4,000 pg/ml (n = 76)	> 4,000 pg/ml (n = 71)	*p*
Age (years)	29.86 ± 3.16	29.95 ± 3.11	29.48 ± 3.17	0.638
Retrieved oocyte number	9.78 ± 4.75	14.22 ± 6.04	19.14 ± 7.18	*0.001*
Embryo/transfer	2.69 ± 0.66	2.93 ± 0.25	2.97 ± 0.23	*0.001*
Pregnancy rate (%)	24.6	14.5	28.2	0.116

Note: Values are means ± SD

Table 3. — *Comparison of variables between the groups according to serum E_2 levels on the day of hCG administration in long GnRH agonist protocol cycles.*

E_2 levels	< 2,000 pg/ml (n = 30)	2,000–4,000 pg/ml (n = 50)	> 4,000 pg/ml (n = 50)	*p*
Age (years)	29.80 ± 3.28	29.44 ± 3.26	29.40 ± 3.27	0.854
Retrieved oocyte number	10.47 ± 4.95	14.12 ± 5.09	19.14 ± 7.93	*0.001*
Embryo/transfer	2.67 ± 0.71	2.92 ± 0.27	3.0 ± 0	*0.004*
Pregnancy rate (%)	26.7	14	26	0.254

Note: Values are means ± SD

sound. hCG was administered when there were at least two follicles >18 mm in diameter. The serum E_2 level was measured on the same day as the injection. Oocyte retrieval was performed 36 hours after hCG administration by ultrasound-guided follicular aspiration. Three days after oocyte retrieval, grade 1 and 2 embryos were replaced. Pregnancy was diagnosed by serum concentrations of β-hCG on day 12 of the embryo transfer. Also, transvaginal ultrasound was performed during sixth week of gestation.

Patient characteristics and outcomes of long GnRH agonist- and GnRH antagonist-protocol cycles were compared. The patients were classified into three groups according to serum E_2 levels measured on the day of hCG injection: < 2,000 pg/ml (Group 1), 2,000–4,000 pg/ml (Group 2), and > 4,000 pg/ml (Group 3). The three groups were compared according to age, number of retrieved oocytes, number of transferred embryos, and pregnancy rates for each of the stimulation protocols.

Data were analyzed with NCSS 2007 and PASS 2008 statistical software programs. The differences between long and antagonist protocols were assessed using Chi-square for categorized variables, and t-test and Mann Whitney U test for continuous variables. Comparisons according to serum E_2 levels on the day of hCG administration were assessed using Chi-square for categorized variables, and one-way ANOVA and Kruskal–Wallis test for continuos variables. The level of statistical significance was defined as $p < 0.05$, with a confidence level of 95%.

Results

Demographic characteristics of the patient population are detailed in Table 1. The long GnRH agonist and GnRH antagonist protocols were performed in 130 and 82 cycles, respectively. The overall clinical pregnancy rate per transfer was 22.2%. The duration of infertility was significantly lower ($p = 0.039$), and serum E_2 level and the number of retrieved oocytes were significantly higher in the long GnRH-agonist protocol group in comparison to the GnRH antagonist-protocol group ($p = 0.001$ and 0.039, respectively). However, there was no statistically significant difference between the pregnancy rates (21.5% vs. 23.2%, $p = 0.781$).

Serum E_2 levels were measured on the day of hCG administration as < 2,000 pg/ml in 65 cycles, 2,000–4,000 pg/ml in 76 cycles, and > 4,000 pg/ml in 71 cycles (Table 2). The number of retrieved oocytes increased in parallel to serum E_2 levels ($p = 0.001$). However, we found no statistically significant difference among groups in the pregnancy rates ($p = 0.116$).

Table 3 shows the ART outcomes of the long GnRH agonist-protocol cycles. Serum E_2 levels were measured on the day of hCG injection as < 2,000 pg/ml in 30 cycles,

Table 4. — *Comparison of variables between the groups according to serum E_2 levels on the day of hCG administration in GnRH antagonist-protocol cycles.*

E_2 levels	< 2,000 pg/ml (n = 35)	2,000–4,000 pg/ml (n = 26)	> 4,000 pg/ml (n = 21)	*p*
Age (years)	29.91 ± 3.10	30.92 ± 2.67	29.67 ± 2.97	0.276
Retrieved oocyte number	9.20 ± 4.57	14.42 ± 7.56	19.14 ± 5.14	*0.001*
Embryo/transfer	2.71 ± 0.62	2.96 ± 0.19	2.90 ± 0.43	0.082
Pregnancy rate (%)	22.9	15.4	33.3	0.349

Note: Values are means ± SD

2,000–4,000 pg/ml in 50 cycles, and > 4,000 pg/ml in 50 cycles. The number of retrieved oocytes increased in parallel to serum E_2 levels ($p = 0.001$). Additionally, there were no significant difference in the pregnancy rates among the groups ($p = 0.254$).

Table 4 shows ART outcomes of GnRH antagonist-protocol cycles. Serum E_2 levels were measured on the day of hCG administration as <2000 pg/ml in 35 cycles, 2,000–4,000 pg/ml in 26 cycles, and > 4,000 pg/ml in 21 cycles. The number of retrieved oocytes increased in parallel to serum E_2 levels ($p = 0.001$). However, there were no significant differences in the pregnancy rates among the three groups ($p = 0.349$).

Discussion

It is known that the success of implantation depends on both endometrial receptivity and embryo quality. The hormone levels in natural cycles are ideal for the development of the embryo and endometrium. However, serum E_2 levels are at least ten times higher in ART cycles [12]. Recent investigations have suggested that high E_2 levels have a detrimental influence on pregnancy outcomes by two different mechanisms. One involves cellular changes in oocytes and the endometrium as a consequence of the alteration of the estradiol–progesterone balance, which decrease the receptivity of the endometrium [13]. The other mechanism is the direct toxic effect of high E_2 levels on embryos, which may negatively influence the implantation process [14]. However, the second mechanism is controversial because some reports have demonstrated that the quality of oocytes and embryos did not appear to be negatively affected by serum E_2 levels [8, 15]. Suboptimal environment for implantation may be due to the gland–stromal dyssynchrony, which shows a deficient secretory endometrial transformation of the endometrium [16]. The color Doppler analysis of periimplantation endometrial perfusion showed that high estradiol concentrations after ovarian stimulation impaired uterine blood flow and implantation [17, 18]. Ma *et al.* stated that the higher levels of estrogen cause the window of uterine receptivity to close rapidly, in contrast to the lower levels of estrogen in which the window of receptivity remains open for an extended period. Uterine refractoriness at high estrogen levels is accompanied by aberrant uterine expression of implantation-related genes [19]. In addition, it is reported that supraphysiologic E_2 concentrations also prevent implantation by augmenting uterine contractility [20].

Joo *et al.* reported a positive correlation between pregnancy rates and serum E_2 levels up to 4,000 pg/ml. However, when serum E_2 levels were higher than 4,000 pg/ml, there was a tendency toward a decrease in pregnancy outcomes [9]. In another study, pregnancy rates achieved a plateau at E_2 levels > 2,500 pg/ml and it not change significantly thereafter [21]. Both reports suggested that serum E_2 levels increase in parallel to oocyte number. However, after the optimal concentration is reached, the increasing quantity of retrieved oocytes does not further improve the implantation rates. When a level higher than the optimal level is reached, the detrimental effect on endometrial receptivity seems to reduce pregnancy rates.

Although most authors have used the long GnRH agonist protocol, Kyrou *et al.* investigated GnRH antagonist-protocol cycles [11]. We aimed to perform both stimulation protocols and compare the impacts of serum E_2 levels on ART outcome. Although the retrieved oocyte number was higher with serum E_2 levels of > 4,000 pg/ml than with serum E_2 levels of 2,000–4,000 pg/ml and < 2,000 pg/ml in both the long GnRH agonist and GnRH antagonist protocols, there was no significant difference in pregnancy rates. In addition, when we compared the long GnRH agonist and GnRH antagonist protocols, they realized that both the oocyte number and serum E_2 levels were higher in the long GnRH agonist-protocol group, whereas age, total dosage of gonadotropin, and day-3 FSH and E_2 concentrations were similar in the two groups. In the total patient population, retrieved oocyte and transferred embryo numbers increased in parallel to serum E_2 levels with no positive effect on pregnancy rates. This supports the theory that high E_2 levels do not have a detrimental effect on embryo development, but after an optimal threshold, higher numbers of oocytes do not improve the embryo implantation rate.

In conclusion, the present study showed that serum E_2 levels on the day of hCG administration do not predict the pregnancy outcome in ART cycles with either long GnRH agonist or GnRH antagonist protocols, although there is a positive correlation between the hormone level and the number of retrieved oocytes.

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