Value of serum estradiol levels on the third day of controlled ovarian hyperstimulation as a predictor of pregnancy success for art

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Summary

Objective:
The objective of the present study is to determine the use of day three estradiol levels after the initiation of controlled ovarian hyperstimulation (COH) to predict pregnancy success in women undergoing COH for ART.

Material and methods:
The study is designed as a two year retrospective study including two-hundred seventy-seven patients undergoing COH for assisted reproduction in an assisted conception unit of a university hospital. The patients were grouped according to the increase of serum estradiol levels at day three after the initiation of COH, compared to baseline serum estradiol levels [one-fold increase in group 1 (n=43), two-fold increase in group 2 (n=86), three-fold increase in group 3 (n=57) and four-fold increase in group 4 (n=91)]. The outcome was evaluated as clinical pregnancy rate together with the number of oocytes retrieved and the number of embryos available for transfer per controlled ovarian hyperstimulation cycle.

Results:
The highest clinical pregnancy rate was found in group 3 (49.1%, p=0.004). The highest number of oocytes were retrieved in group 4 (12.6±7.1, p=0.001). The fertilization rates in group 1, group 2, group 3 and 4 were found to be 75%, 78%, 79% and 74%, respectively. The mean number of ampoules used were 42.6±9.6, 35.9±12.1, 27.7±9.7 and 26.2±8.1, respectively.

Conclusion:
As a conclusion, in COH cycles, a three-fold increase of serum estradiol level on the day three of COH predicts the best clinical pregnancy rate.

Keywords:
Assisted reproductive technology, estradiol, oocyte, controlled ovarian stimulation

Introduction

Controlled ovarian hyperstimulation (COH) is frequently used in assisted reproduction techniques (ART). Gonadotropins are used alone or in different combinations to enhance the growth and maturation of multiple oocytes. It is essential to recruit a greater number of follicles to improve the chance of fertilization and to allow an increased number of embryos for transfer to obtain acceptable success rates. The monitoring of COH in ART aims to evaluate whether the response to exogenous gonadotropins is sufficient to obtain an adequate number of mature oocytes. The second aim is to determine the optimal time for the final oocyte maturation by human chorionic gonadotropin administration. Currently, COH is monitored by repeated pelvic ultrasonography or serum estradiol measurements. It is believed that transvaginal sonographic findings reflect growth, whereas serum estradiol levels primarily detect functional activity of follicles.

The ability to predict ovarian response early in the course of COH could provide vital information to patients on successful ovulation induction and subsequent pregnancy. Many ovulation induction protocols include the monitoring of serial serum estradiol levels. Use of the serum estradiol levels detected after the initiation of gonadotropin stimulation may act as an early marker of ovarian responsiveness which may help in deciding whether to proceed with the ongoing cycle (1-4).

Several investigators have evaluated various hormonal and sonographic variables during COH to determine the prognosis for pregnancy for that cycle (1-4). The aim of the present study is to determine the use of day three estradiol levels after the initiation of COH to predict pregnancy success in women undergoing controlled ovarian hyperstimulation for ART.

Material and Methods

Two-hundred seventy-seven patients who had undergone intracytoplasmic sperm injection and embryo transfer treatment between 2000-2001 at Ege University Family Planning, Infertility Research and Treatment Center were enrolled into the study. Forty years and older patients were not included in the study because the negative effect of age on spontaneous pregnancy loss.

Desensitization with long protocol was started in the previous luteal phase with daily administration of triptorelin (Decapeptyl® 0.1 mg, Ferring, Kiel, Germany). In short protocol, desensitization was started just before or simultaneously with the initiation of gonadotropin stimulation in the follicular phase with daily administration of triptorelin (Decapeptyl® 0.1 mg, Ferring, Kiel, Germany). If the serum estradiol concentrations were <70 pg/mL and transvaginal sonography revealed no ovarian cyst >15 mm in diameter, controlled ovulation hyperstimulation with hMG (Pergonal®, Serono, Aubonne, Switzerland; Humegon®, Organon, Oss, Netherlands), urinary FSH (Metrodin®, Serono, Aubonne, Switzerland), recombinant FSH (Puregon®, Organon, Oss, Netherland or Gonal F®, Serono, Aubonne, Switzerland) was commenced. Meanwhile, triptorelin 0.1 mg/day s.c. was continued until the day of hCG administration.

The dose of gonadotropin hormone was individualized according to the patient's age, baseline hormone concentrations, and previous stimulation history or response to stimulation. Cycles were monitored by transvaginal sonography and serum estradiol levels. Follicular maturation was completed by the administration of 10.000 IU hCG (Pregnyl® 5000 IU, Organon; Profasi® 5000 IU, Serono, Bari, Italy). Endometrial thickness was measured at the uterine fundus in longitudinal axis by transvaginal sonography. Oocyte retrieval was performed using transvaginal sonographic guidance 36 hours after hCG administration. Intracytoplasmic sperm injection procedure was accomplished as described previously (5).

Luteal phase support was provided by the administration of progesterone vaginal suppositories 800 mg daily (Utrogestan®, Iscovesco, Paris, France) and hCG (1500-2000 IU) administration on days +1, +4, +7 and +9 after embryo transfer. A clinical pregnancy was defined when serum hCG concentrations reached >1000 IU/mL and intrauterine gestational sac with fetal heartbeat was detected by transvaginal sonography.

Serum collected on cycle day 3 following COH cycle was assayed for estradiol level with an automated system called Automated Cheiluminescense (ACS) 180; Chiron diagnostic corporation). The outcome was evaluated as clinical pregnancy rate together with the number of oocytes retrieved and the number of embryos available for transfer per controlled ovarian hyperstimulation cycle. The patients were grouped according to the increase of serum estradiol levels at day three after the initiation of COH, compared to baseline serum estradiol levels [one-fold increase in group 1 (n=43), two-fold increase in group 2 (n=86), three-fold increase in group 3 (n=57) and four-fold increase in group 4 (n=91)].

Statistical analysis was performed by SPSS package program (Chicago, IL). The values were expressed as mean ± standard deviation. The means were compared by one-way analysis of variance. The percentages were compared by chi-square test. P value lower than 0.05 was accepted as significant.

Results

The clinical pregnancy rates in group 1, group 2, group 3 and 4 were 18.6% (8/43), 25.5% (22/86), 49.1%(28/57) and 29.6%(27/91), respectively (p=0.004) (Table 1). The mean age, the mean follicle stimulating hormone level, the mean number of ampules used, maximum level of estradiol, the number of oocytes retrieved and fertilization rates were demonstrated in Table 2. The highest clinical pregnancy rate was found in group 3 (49.1%, p=0.004). The highest number of oocytes were retrieved in group 4 (12.6±7.1, p=0.001). The fertilization rates in group 1, group 2, group 3 and 4 were found to be 75%, 78%, 79% and 74%, respectively (Table 2). The mean number of ampoules used were 42.6±9.6, 35.9±12.1, 27.7±9.7 and 26.2±8.1, respectively.

Discussion

Hershlag et al (6) examined 18 IVF cycles in which the estradiol level after five days of gonadotropin stimulation was <50 pg/mL. They reported a very poor outcome of these cycles despite increasing the dose of human menopausal gonadotropin (hMG) during the course of stimulation. It is clear that, the follicular phase variables used for cycle cancellation in hMG-stimulated ART cycles cannot be used in gonadotropin releasing hormone analog/hMG cycles (7). However, Phelps et al (4) demonstrated that low day four serum estradiol levels in COH cycles using luteal phase leuprolide acetate predict higher cancellation rates because of poor response.

Phelps et al (4) also reported that estradiol levels obtained on the fourth day of gonadotropin therapy are highly predictive of COH and pregnancy outcome in cycles using luteal phase leuprolide acetate. The clinical pregnancy and delivery rates for cycles with day four estradiol levels of >75 pg/mL were 42.3% and 32.4%, respectively. These rates differed significantly form those for cycles with day four estradiol levels of <75 pg/mL, which were only 9.1% and 6.8%, respectively. The number of oocytes retrieved and the number of embryos available for transfer for cycles with day four estradiol levels of >75 pg/mL also differed signifi-

cantly from those for cycles with day 4 estradiol levels of <75 pg/mL.

In COH cycles using luteal phase buserelin, low initial serum estradiol concentrations are associated with poor outcome. Khalaf et al (8) classified the patients into four groups according to serum estradiol levels on the sixth day of COH: group A (estradiol level <50 pg/mL), group B (estradiol level 51-100 pg/mL), group C (estradiol level 101-200 pg/mL), and group D (estradiol level >200 pg/mL). Group A experienced the highest cancellation rates (65.1%) and lowest pregnancy rates (7.8%) despite requiring significantly more hMG ampoules (47.8±1.7). The cancellation rate was higher (75.1%) and no pregnancy occurred in a subset of group A in whom COH was initiated with >3 ampules (225 IU) of gonadotropins.

The poor outcome of cycles with low initial estradiol levels could be explained by the direct effect of estradiol on modulating oocyte function (9). It may also result from lack of an adequate estrogenic intrafollicular milieu. This milieu is important for the oocyte's developmental competence, cytoplasmic maturation, including activation, synthesis of the male pronucleus growth factor, and preimplantation development (10).

In the present study, the highest clinical pregnancy rate was found in group 3 (49.1%, p=0.004). The highest number of oocytes were retrieved in group 4 (12.6±7.1, p=0.001). The fertilization rates in group 1, group 2, group 3 and 4 were found to be 75%, 78%, 79% and 74%, respectively. The mean number of ampoules used were 42.6±9.6, 35.9±12.1, 27.7±9.7 and 26.2±8.1, respectively.

There is still debate on the impact of high estradiol levels on the day of hCG administration on implantation/pregnancy rates in patients undergoing ART. Sharara et al (11) concluded that peak estradiol levels >3000 pg/mL were not detrimental to ART outcome in 106 patients undergoing the first ART cycle and suggested that the threshold might be >5.000 pg/mL. Ng et al (12) evaluated 1.122 women of age <40 years undergoing their first ART cycle. Estradiol levels on the day of hCG were categorized into three groups: group A <10.000 pmol/L; group B 10.000-20.000 pmol/L; and group C >20.000 pmol/L. In fresh cycles, group A had significantly lower pregnancy rate per transfer (16.2% versus 23.7%, respectively, p=0.005) and implantation rate (8.7% versus 11.7%, respectively, p=0.037), when compared with group B. Pregnancy rate per transfer in group C was significantly lower than group B (12.1% versus 23.7%, p=0.049) and group C had the lowest implantation rate (6.4%).

The mechanism of high estradiol levels leading to impaired implantation is unclear. In a recent study by Basir et al (13) glandular and stromal components of the endometrium were assessed by morphometric criteria to investigate whether high estradiol concentrations after COH affect endometrial development around the time of implantation. The women with estradiol concentration >20.000 pmol/L showed delayed glandular maturation and advanced stromal morphology, whereas the women with estradiol concentration <20.000 pmol/L demonstrated synchronous development of glandular and stromal features. The effect of excessively high estradiol concentrations was explained by quantitative evaluation of the endometrial biopsies as gland-stromal dyssynchrony, which indicated a deficient secretory transformation of the endometrium. This represents a suboptimal environment for implantation.

Unlike FSH levels and baseline estradiol concentrations, estradiol levels obtained early after the initiation of COH directly reflect follicular activity and ovarian responsiveness to the ongoing regimen of gonadotropin stimulation. In conclusion, a three-fold increase of serum estradiol level on the day 3 of COH predicts the best clinical pregnancy rate.

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Table 1
The clinical pregnancy rates in group 1, group 2, group 3 and group 4.

Groups	Number of clinically pregnant patients n	Total number of patients n	Percentage %
1	8	43	18.6
2	22	86	25.5
3	28	57	49.1
4	27	91	29.6
Total	85	277	30.6

The increase of serum estradiol levels at day three after the initiation of controlled ovarian hyperstimulation compared to baseline serum estradiol levels (one-fold increase in group 1, two-fold increase in group 2, three-fold increase in group 3 and four-fold increase in group 4).

Table 2
The mean age, the mean follicle stimulating hormone level, the mean number of ampules used, maximum level of estradiol, the number of oocytes retrieved and fertilization rates in groups 1-4.

	Group 1	Group 2	Group 3	Group 4	P value
Age	30.7±2.9	29.7±3.6	30.2±3.2	29.6±3.9	0.29
FSH (IU/mL)	2.2±1.6	2.9±1.8	3.2±2.0	3.3±2.3	0.02
Ampouls used	42.6±9.6	35.9±12.1	27.7±9.7	26.2±8.1	0.001
Maximum E2 (pg/mL)	1389±1121	1371±955	1515±912	1942±1359	0.004
Oocytes retrieved	8.7±6.0	9.5±6.2	9.2±4.8	12.6±7.1	0.001
Fertilization rate	75%	78%	79%	74%	

FSH: Follicle stimulating hormone, E2: Estradiol
The increase of serum estradiol levels at day three after the initiation of controlled ovarian hyperstimulation compared to baseline serum estradiol levels (one-fold increase in group 1, two-fold increase in group 2, three-fold increase in group 3 and four-fold increase in group 4).