Estradiol Level on Day 9 as a Predictor of Risk for Ovarian Hyperresponse During Controlled Ovarian Hyperstimulation¹

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Purpose: To investigate the estradiol (E_2) level in the mid-follicular phase during controlled ovarian hyperstimulation (COH) and evaluate it as a predictor of a high risk for ovarian hyperresponse.

Methods: From January 1996 to October 2001, the records of a total of 146 patients undergoing 164 COH cycles were retrospectively reviewed. All patients received the long protocol of GnRH agonists from the previous mid-luteal phase and then hMG or FSH from day 3 of the menstrual cycle. The E_2 level was evaluated on day 9. Ovarian hyperresponse was defined as 1) an E_2 level on the day of hCG injection was >4000 pg/mL, or 2) the necessity for coasting during COH to decrease the risk of ovarian hyperstimulation syndrome (OHSS).

Results: Of the 52 cycles in which day 9 E_2 level was >800 pg/mL, 29 (55.8%) fulfilled the criteria for ovarian hyperresponse. None of patients whose day 9 E_2 level was <300 pg/mL met the criteria for hyperresponse. The pregnancy rate in the groups with day 9 E_2 level <300 pg/mL was 42.9%; for an E_2 level = 300–800 pg/mL, 49.2%; and for an E_2 level >800 pg/mL, 32.7%. The corresponding implantation rates were 18.8, 28.0, and 17.0%. The E_2 level on day 9 did not correlate with clinical pregnancy rates or implantation rates.

Conclusions: A high E_2 level in the mid-follicular phase was predictive of patients with a high ovarian response. An E_2 level on day 9 of menstrual cycle of >800 pg/mL suggests an increased risk for ovarian hyperresponse, and appropriate management should be instituted to decrease the risk of OHSS.

KEY WORDS: Controlled ovarian hyperstimulation; estradiol; ovarian hyperresponse.

INTRODUCTION

The ovarian hyperstimulation syndrome (OHSS) is a serious, potentially life-threatening complication of

infertility treatment. Although the underlying mechanism of OHSS remains uncertain, most episodes follow controlled ovarian hyperstimulation (COH), a procedure routinely performed to increase the number of oocytes available for assisted reproductive techniques (ART) (1). The incidence of OHSS is influenced by the clinician's understanding of prevention of the syndrome (2–5). Because it is an iatrogenic condition, it is the physician's responsibility to try to avoid its occurrence. However, despite significant improvement in monitoring follicular development by serial estradiol (E_2) assay and ultrasound, severe OHSS has not been eliminated.

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Many strategies to prevent OHSS depend on the ability to predict the probability of OHSS occurring in an individual patient. Many conditions predispose to OHSS, such as polycystic ovarian syndrome (PCOS) (3,6), hypothyroidism (4), lean habitus, and young age (2,4,6,7). However, these do not reflect ovarian responsiveness in any individual cycle, and reliable prediction of OHSS is not possible (5,6,8). Attention has therefore been focused on identifying risk factors that are manifest during COH. It is well documented that E₂ variation is a good predictor of ovarian sensitivity to gonadotropins (9), and E₂ measurements are routinely used to calibrate the gonadotropin dose in conjunction with data obtained by ultrasound (10). The predictive value of measuring early cycle serum E₂ to estimate cycle outcome for traditional in vitro fertilization (IVF) cycles has been investigated (11,12).

There is no published data on the use of mid-follicular E₂ levels to predict ovarian hyperresponse and OHSS. The aim of the present study was to evaluate the clinical utility of mid-follicular phase serum E₂ levels during COH in order to identify ovarian hyperresponders who are at high risk for OHSS.

MATERIALS AND METHODS

This study was approved by our local institutional review board. The records of patients undergoing IVF between January 1996 and December 2001 were retrospectively selected from our database. Patients who were older than 40 years of age, who had undergone previous uterine or ovarian surgery, or whose day 3 FSH level was >15 IU/mL were excluded in order to eliminate known confounding variables such as possible poor ovarian reserve and poor uterine receptability. Patients who were suspected of having PCOS by history and ultrasonography were also excluded because they are often high responders to COH. Therefore, all patients evaluated in the study had no recognized prior disposition to either high or low ovarian response to COH.

All patients had received the long protocol of pituitary desensitization with the GnRH analogue, leuprolide acetate (Lupron; Abbott laboratories, Illinois, USA) from the mid-luteal phase of the previous cycle to the day of human chorionic gonadotropin (hCG, Pregnyl; Organon, Oss, The Netherlands) injection. COH was achieved by combination of 3–4 ampoules of human menopausal gonadotropin (hMG, Pergonal; Serono, Rome, Italy) and follicle stimulating hormone (FSH, Metrodin; Serono, Rome, Italy),

starting from day 3 of the menstrual cycle. After 6 days of COH with gonadotropins, the E₂ level was determined from venous blood on the morning of day 9 of the menstrual cycle.

The level of E₂ in serum was measured with a radioimmunoassay kit (Coat-A-Count, Diagnostic Product Corporation, Los Angeles, USA). The sensitivity, defined as the 95% coefficient of variation of repeated blank measurements, was 8 pg/mL. The intraassay and interassay coefficients of variation for mean E₂ concentrations were 7.0 and 8.1%, respectively.

If the E₂ concentration was over 3000 pg/mL and the mean diameter of the leading follicle was larger than 16 mm, the COH procedure was allowed to “coasting,” i.e., gonadotropins were discontinued and hCG administration was deferred until the E₂ level declined.

When two or more leading follicles had mean diameters of >18 mm in two dimensions, 10000 units of hCG were given. Transvaginal ultrasound-guided ovum retrieval was performed under general anesthesia 34 h following hCG administration. The E₂ level on the day of hCG injection was recorded.

The outcome of interest was ovarian hyperresponse, a documented risk factor for OHSS. We used the following objective criteria to define this outcome: 1) an E₂ level on the day of hCG injection >4000 pg/mL, or 2) the institution of coasting to decrease the risk of OHSS.

Transcervical or transfallopian embryo transfer was carried out 48–72 h after oocyte retrieval. The luteal phase was supported by three injections of 2500 units of hCG every third day, beginning 48 h after oocyte retrieval, or 50 mg of progesterone (progesterone, Tai Yu Chemical Pharmaceutical Co., Shing Ju, Taiwan) administered intramuscularly daily, beginning the day of ovum pick up. Clinical pregnancy was documented by transvaginal ultrasonographic identification of an intrauterine gestational sac.

The E₂ level on day 9 of menstrual cycle and the subjects' ovarian response was analyzed by the chi-square test, ANOVA test. Statistical analysis was conducted using the SPSS software. A *p* value of <0.05 was considered statistically significant.

RESULTS

A total of 146 patients undergoing 164 cycles of COH fulfilled the inclusion criteria. According to the E₂ level on day 9 of menstrual cycle, they were

Table I. Patient Characteristics

	E ₂ level on day 9 (pg/mL)			p value
	<300	300–800	>800	
No. of patients	46	58	47	
No. of cycles	49	63	52	
Age (years)	31.9 ± 4.1	32.3 ± 3.7	31.9 ± 3.1	NS
Duration of infertility (years)	3.7 ± 3.1	3.0 ± 2.2	3.4 ± 2.9	NS
Gonadotropins used before day 9 (ampoules)	19.2 ± 3.9	19.9 ± 3.9	20.4 ± 3.7	NS

Note. Values are means ± SD. NS = not significant on ANOVA.

divided into three groups: <300 pg/mL (46 patients, 49 cycles), 300–800 pg/mL (58 patients, 63 cycles), and >800 pg/mL (47 patients, 52 cycles). Among the three groups, there were no significant differences in mean age, duration of infertility, or the total dose of gonadotropins used before day 9 of the menstrual cycle (Table I).

Table II shows the relationship between the E₂ level on day 9 and risk of ovarian hyperresponse. When E₂ was >800 pg/mL, 29 cycles (55.8%) fulfilled the definition of ovarian hyperresponse, while no patient whose E₂ level on day 9 was <300 pg/mL was hyperresponder. Therefore, the E₂ level on day 9 of the menstrual cycle provided useful information for predicting ovarian hyperresponders with the risk for OHSS.

Table III shows that, although a lower total dose of gonadotropins for COH were given to patients with a higher E₂ level on day 9, these women still had a significantly higher maximal E₂ level during COH, more dominant follicles, more oocytes retrieved, and more embryos available. E₂ level on day 9 did not significantly correlate with clinical pregnancy rates or implantation rates.

Table II. E₂ Level on Day 9 and Risk for Ovarian Hyperresponse^a

	E ₂ level on day 9 (pg/mL)			p value
	<300	300–800	>800	
No. of cycles	49	63	52	
Ovarian hyperresponse	0	11 (17.5%)	29 (55.8%)	<0.01 ^b

^aDefined as 1) an E₂ level on the day of hCG injection >4000 pg/mL, or 2) the institution of coasting to decrease the risk of OHSS.

^bChi-square test.

DISCUSSION

Severe OHSS is a dramatic iatrogenic complication of COH. A satisfactory ART program is critically dependent on optimizing COH protocols, not only to achieve good quality embryos and a high pregnancy rate, but also to minimize medical complications such as OHSS. Therefore, it is very important to individualize stimulation protocols with respect to ovarian response.

The main determining factor in the development of OHSS appears to be ovarian predisposition (3). Many risk factors for or predictors of possible OHSS have been investigated. Blankstein *et al.* used the antral follicle count to predict OHSS (13). Danninger *et al.* suggested that baseline ovarian volume by three-dimensional ultrasonography was a good predictor (14). However, these are baseline parameters and do not provide direct information about the responsiveness of the ovaries to the exogenous gonadotropins during COH. They do not always accurately reflect the degree of stimulation, cycle outcome, or intercycle fluctuations of ovarian response in repeated COH cycles.

Many COH protocols include the monitoring of serial E₂ levels. Fanchin *et al.* showed that, with administration of a fixed dose of exogenous gonadotropins, the relative increment of serum E₂ concentration is a better predictor of ovarian response than the day 3 FSH value (9). However, their focus was detection of poor ovarian responders, and they did not use it to assess the risk of ovarian hyperresponse and OHSS.

The E₂ level on day 9 of the menstrual cycle assesses the patient's condition part way through a COH protocol, which we believe provides a more dynamic view of that particular cycle. Patients enrolled in our study had no prior known risk factors for OHSS. They all received an identical protocol of pituitary downregulation and fixed dose of gonadotropins in the first 6 days of COH, so that different levels of E₂ on day 9 demonstrated the variability of ovarian response between subjects during any particular cycle.

COH for IVF actually entails a mild form of OHSS (1,3,6). Using subjective parameters to define OHSS is fraught with difficulty. An E₂ level on the day of hCG injection >4000 pg/mL has been shown to be a marker for high risk of OHSS (1,2). We defined these patients as ovarian hyperresponders. An E₂ level >3000 pg/mL before the day of hCG injection also indicates a high risk of OHSS (4), as the E₂ level would be expected to continue rising with continued ovarian stimulation. In this circumstance, coasting is

Table III. E₂ Level on Day 9 and IVF Outcome

	E ₂ level on day 9 (pg/mL)			p value
	<300	300–800	>800	
No. of patients	46	58	47	
No. of cycles	49	63	52	
Gonadotropins used after day 10 (ampoules)	16.4 ± 5.7	12.6 ± 5.5	8.3 ± 3.3	<0.001
Total gonadotropins used (ampoules)	35.5 ± 7.4	32.5 ± 7.0	28.7 ± 6.1	<0.05
Maximal E ₂ level (pg/mL)	1534.1 ± 803.1	2805.4 ± 1242.2	4496.0 ± 2206.7	<0.001
No. of dominant follicles	7.0 ± 3.4	9.2 ± 3.3	12.4 ± 4.3	<0.01
No. of oocytes retrieved	7.5 ± 3.6	11.9 ± 5.2	16.0 ± 5.2	<0.001
No. of mature oocytes	6.8 ± 3.2	10.4 ± 4.6	14.2 ± 4.6	<0.001
No. of embryos available	3.9 ± 2.4	5.2 ± 3.3	7.2 ± 3.6	<0.05
No. of embryos transferred	3.0 ± 1.4	3.4 ± 1.2	3.6 ± 1.0	NS ^a
Clinical pregnancy	21 (42.9%)	31 (49.2%)	17 (32.7%)	NS ^b
Implantation rate	18.8%	28.0%	17.0%	NS ^b

Note. Values are means ± SD.

^aNS = not significant on ANOVA.

^bNS = not significant with chi-square test.

recommended (4,15). These patients were also defined as ovarian hyperresponders in our study.

If the E₂ level on day 9 was >800 pg/mL, in 55.8% of those cycles, the patients subsequently fulfilled the criteria for ovarian hyperresponse. No patient with a day 9 E₂ level of <300 pg/mL met the criteria. These data suggest that E₂ levels >800 pg/mL on day 9 of the menstrual cycle are good predictors of ovarian hyperresponse. We recommend decreasing the dose of gonadotropins at that point in order to tailor the ovarian response.

Delvigne *et al.* (8,16) suggested that a steep rise of E₂ during COH increases the likelihood of OHSS, but they did not clearly define objective clinical data. Many physicians ignore the steepness of the E₂ slope or do not have objective criteria to evaluate the rate of rise of E₂ during COH. The advantage of an E₂ level on day 9 is that it predicts if a woman will be hyperresponder in that particular cycle.

Many investigators have tried to estimate the risk of OHSS with ultrasonographic evaluation, including ovarian volumetry (14), antral follicle count (13,17), and intraovarian vascular resistance (18). In our view, since E₂ synthesis is associated with follicular development, it directly reflects ovarian function, rather than anatomy, during COH. Therefore, it offers an easily assessable dynamic test of ovarian function that can be used as an additional tool for estimating OHSS risk.

Although all patients in our study received the same dose of gonadotropins initially, the dose was modified according to the day 9 E₂ level due to safety and eth-

ical concern. This is why patients with a higher E₂ level on day 9 received a lower total dose after day 10. Navot *et al.* and Delvigne *et al.* also found that women who went on to develop OHSS had a significantly lower ratio of gonadotropin dose to duration of stimulation (8,12). In spite of the dose adjustment in our patients, 55.8% of the cycles with a high E₂ level on day 9 subsequently led to ovarian hyperresponse. The number might have been even higher had we not tailored the dose.

Table III shows that, in patients with high E₂ levels on day 9, the maximal E₂ during COH, the number of dominant follicles, the number of oocytes retrieved, and the number of embryos available were significantly higher. These data are comparable with the findings of Phelps *et al.* that patients with a high proliferative phase E₂ tend to have more oocytes (11). However, since we restricted the number of embryos transferred, the day 9 E₂ level did not correlate with the clinical pregnancy rates or implantation rates. Therefore, while a high ovarian response does not necessarily give a better pregnancy rate, it does increase the risk of complications of OHSS. It is very important to adjust COH strategy to achieve a high pregnancy rate while minimizing the incidence of complications.

No single test of ovarian response is precise enough to merit total reliance on the results. Because of assay variation, our cutoff of 800 pg/mL should be used only as a guideline. It is important that each center establish its own threshold values for protocol modification.

CONCLUSION

E₂ level on day 9 of menstrual cycle can be used as a predictor of ovarian hyperresponse, which confers an increased risk of OHSS. If the E₂ level on day 9 is >800 pg/mL during any particular COH cycle, the dose of gonadotropins should be adjusted in order to decrease the risk of ovarian hyperresponse.

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