CLINICAL STUDY

Predicting the Ovarian Response in ART Cycles Using Rate of Increase in Serum Estradiol on Day Six of Controlled Ovarian Hyperstimulation

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Abstract

Objective: Although, earlier studies have evaluated the significance of various estradiol patterns during controlled ovarian hyperstimulation (COH), their prognostic significance remains unclear. The objective of this study is to investigate the rate of increase in estradiol (ROI-E2) during COH and evaluate it as a predictor of ovarian response.
Materials and Methods: In this retrospective study, 130 patients undergoing COH for IVF/ICSI cycles were reviewed. The E2 level during COH was evaluated on day 1, 5, 6, 7 & 8 and the ROI-E2 was determined. Age, basal FSH, number of mature follicles, number and maturity of oocytes, and incidence of ovarian hyperstimulation syndrome (OHSS) were recorded and their relationship with ROI-E2 was determined.
Results: Patients with elevated basal FSH have shown poor ROI-E2 on day six and a significant negative correlation was observed. Similarly, patients with advanced age group demonstrated poor ROI-E2 on day six. The average ROI-E2 on day six was significantly higher in patients who developed OHSS than non-OHSS group. The number of follicles, oocytes and maturity of the oocytes were maximum among the patients with higher ROI-E2 on day six.
Discussion: In a COH cycle, there is significant predictive association between ROI-E2 on day six and good ovarian response and hyper-response.

Keywords: ovarian response, rate of increase in serum estradiol, assisted reproductive technology (ART)

Özet

Yardımla Üreme Teknikleri Sikluslarında Ovaryen Yanıtın Prediksiyonunda Altıncı Gün Serum Östrojen Artış Oranının Önemi

Amaç: Her ne kadar önceki çalışmalarda kontrollü ovaryen hiperstimülasyon esnasında değişik östrojen paternlerinin önemi araştırılmış olsa da, prognostik önemleri halen bilinmemektedir. Bu çalışmanın amacı ovaryen yanıtın prediksiyonunda östrojen artış oranının (ROI-E_2) önemini araştırmaktır.
Materyal ve Metot: Bu retrospektif çalışmada, IVF/ICSI siklusu için kontrollü ovaryen hiperstimülasyon yapılan 130 hasta tekrar gözden geçirilmiştir. Kontrollü ovaryen hiperstimülasyon esnasında, serum E_2 düzeyi 1., 5., 6., 7. ve 8. günlerde incelenmiş ve ROI-E_2 değerleri hesaplanmıştır. Yaş, bazal FSH, matür folikül sayısı, oosit matürasyonu ve sayısı ile ovaryen hiperstimülasyon sendromu (OHSS) insidansı rapor edilmiş ve bunların ROI-E_2 ile ilişkileri araştırılmıştır.
Sonuç: Artmış bazal FSH düzeyi olan hastalar, altıncı günde zayıf ROI-E_2 göstermiş ve bu hastalarda anlamlı bir negatif korelasyon tespit edilmiştir. Ayrıca, ileri yaştaki hastalarda da zayıf ROI-E_2 değerleri 6. günde gözlenmiştir. Altıncı gündeki ortalama ROI-E_2 değerleri, OHSS olan hastalarda olmayanlara göre anlamlı olarak yüksek bulunmuştur. Folikül sayısı, oosit sayı ve matürasyonu, altıncı günde yüksek ROI-E_2 olan hastalarda yine anlamlı olarak yüksektir.
Tartışma: Kontrollü ovaryen hiperstimülasyonda altıncı günde elde edilen ROI-E_2 değerleri ile iyi ve aşırı ovaryen yanıt arasında anlamlı bir prediktif ilişki mevcuttur.

Anahtar sözcükler: ovaryen yanıt, serum östradiol artış oranı, yardımla üreme teknikleri

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Introduction

The ability to predict ovarian response early in the course of ovulation induction would provide vital information by forecasting the likelihood of successful ovulation induction, subsequent pregnancy and also other related medical complications like ovarian hyperstimulation syndrome (OHSS). Conventionally, the controlled ovarian hyperstimulation (COH) is monitored by serum estradiol (E_2) levels and pelvic ultrasonography. It is believed that ultrasonographic findings reflect follicle growth, whereas serum E_2 levels primarily detect functional activity of follicles (1). Although, earlier studies have evaluated the significance of serum E_2 levels, E_2 curve patterns, E_2 level per follicles, absolute peak levels, maximal production of E_2 levels per follicle, estradiol patterns during gondaotropin stimulation to predict ovarian responsiveness to COH (2-6), their prognostic significance during COH remains unclear.

The estimation of serum estradiol levels early after the initiation of gonadotropin stimulation could provide an early marker of ovarian response. It has been proven that advanced age of infertile patients results in low response to ovarian stimulation, decrease in E_2 production and a poor prognosis for pregnancy (7-9). Similarly, elevated day 3 FSH levels are associated with lower E_2 levels on the day of hCG administration and with lower numbers of retrieved oocytes (10).

Earlier studies have shown that estimation of day 3 and 4 estradiol level has a prognostic significance in the *in vitro* fertilization cycles (8,11). Furthermore, it has been reported that low serum estradiol concentrations after five days of COH for *in vitro* fertilization are associated with poor outcome (12). In contrast, a significant decreases in pregnancy and implantation rates were observed with higher oestradiol concentrations in the follicular phase (13).

In the present study, we calculated the rate of increase in serum E_2 (ROI-E_2) from each patient undergoing ART at different days starting from the fifth day of COH and evaluated its usefulness in predicting ovarian response. To our knowledge, there is no report on the rate of estradiol increase in predicting ovarian response although estradiol pattern in COH has been well studied. We hypothesized that ROI-E_2 may be more specific, easier to calculate and are independent of the day of measurement. Hence it is expected to predict better than a single measurement of serum estradiol at different days of COH. The primary objectives of our study were therefore; 1) to determine ROI-E_2 at different days of COH 2) to investigate the relationships between ROI-E_2 and age, basal FSH level, number of mature follicles developed, number and quality of oocytes retrieved and 3) to find out the incidence of OHSS in these subjects and its association with ROI-E_2.

Materials and Methods

Subjects

In this retrospective study, the data on total of 130 patients underwent ART treatment in our university infertility centre (Division of Reproductive Medicine, Kasturba Medical College) between 2002-2004 were collected. Ethical approval prior to the commencement of this study was obtained from the institutional ethical committee.

Controlled ovarian hyperstimulation and oocyte pick up

All stimulation protocols during this period involved down regulation with GnRH analogue (Buserlin, Aventis Pharma) starting from luteal phase of the previous cycle (long protocol) followed by gonadotropin therapy after ensuring the adequate down regulation (E_2 levels <50 pg/ml, endometrium <5mm). Only recombinant FSH (Gonal F, Serono) was used and the dose was individualized on the basis of patient age, ovulation history, and prior response to COH (the average starting dose is 250 IU). The dose of gonadotropin was adjusted after day six on the basis of serum estradiol level and ultrasound findings. The follicular development was further monitored using ultrasonography and serum E_2. Follicular maturation was triggered by administering hCG (Profasi 10 000 IU, Serono) when at least three follicles reached 18 mm in diameter. Ultrasound guided transvaginal oocyte retrieval was performed 35 h after hCG administration. The data including the age, number of follicles aspirated, number and maturity of the oocytes and the incidence of OHSS were collected for each patient. The OHSS was diagnosed as mild, moderate and severe form (14).

Hormone estimation

The FSH and E_2 measurement was done using electrochemiluminescence immunoassay kit (Roshe Diagnostics, GmbH). The assay was performed using autoanalyser (Hitachi). The baseline serum FSH level was determined on day 1, where as serum E_2 was estimated on day 1, 5, 6, 7 and 8 of the follicular phase. The ROI-E_2 for different days was calculated as follows:

$$\text{Rate of increase (ROI)} = \frac{E_2 \text{ level on the day of ROI to be determined-previous } E_2 \text{ level}}{\text{Time period in days}}$$

For example, to determine the

$$\text{ROI on day 6} = \frac{E_2 \text{ level on day 6-}E_2 \text{ level on day 1}}{5}$$

Statistical analysis

Results are expressed as means ±SEM. Statistical significance was determined using Student t-test, Chi-square test or one-way analysis of variance (ANOVA) and $p<0.05$ was considered significant. Microcal Origin 6.0 was used for statistical analysis.

Results

One hundred thirty patients recruited for IVF/ICSI program were superovulated after down regulation. Seventeen cycles were cancelled either due to poor response to stimulation or non-availability of embryos for transfer. The average age of the study group was 31.82±0.38 years and the

basal mean FSH value was 6.81±0.25 mIU/ml. The mean basal serum E_2 level on the day of starting gonadotropin was 42.91±2.28 pg/ml and the average number of mature follicles observed on day of oocyte recovery was 13.77±0.89. On oocyte aspiration, we retrieved average 7.160±0.44 oocytes among which 4.91±0.34 were found mature (metaphase II).

Relationship between patient age, basal FSH and ROI-E_2

The basal FSH level has shown to influence the ROI-E_2 during the course of COH. The group of patients with basal FSH<4.0 (group 1) showed a significant increase in ROI-E_2 at all the intervals studied (data not shown) and the mean ROI-E_2 in this group on day 6 was approximately 220 (Figure 1A). The mean ROI-E_2 of the patients with the basal FSH between 5-10 (group 2) was around 150 and we did not find statistical significance between these groups. However, patients with >10 basal FSH (group 3) showed a significant decline in ROI-E_2 compared to group 1 and 2 ($p<0.005$ and $p<0.01$ respectively) as shown in Figure 1A. We performed correlation analysis to find out the association between ROI-E_2 and basal FSH level. The basal FSH level has shown a good correlation (r: -0.4) with the ROI-E_2 estimated on day 6 (Figure 1B) but not found significance with ROI-E_2 on day 5 (data not shown).

To understand the relationship between the ROI-E_2 and the patient's age, we categorized the subjects based on their age. The group-1 comprised age below 25 years, group-2 between 26-35 years and the group-3 above 36 years. The mean ROI-E_2 for group-1 was around 190 on day 6 whereas the group 2 and 3 had the average ROI-E_2 of 157 and 146 respectively which did not show any statistical difference (Figure 1C). The correlation analysis for all the time intervals also showed no significant association between age and ROI-E_2 day 6 (Figure 1D) and the other intervals studied.

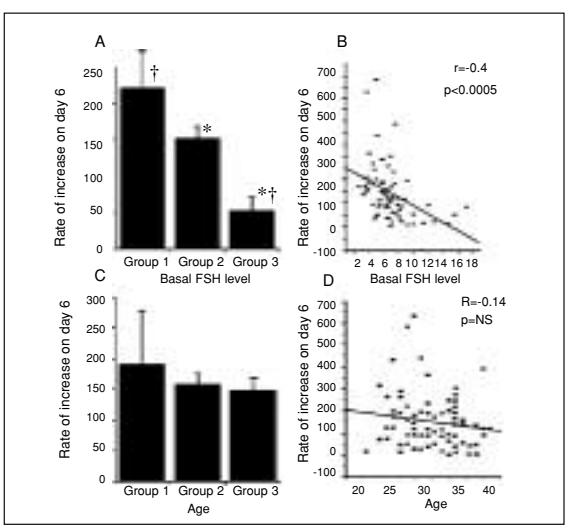

Figure 1. A. Comparison of the ROI-E_2 on day six of COH and basal FSH level ($p<0.005$ between † and 0.01 between *. **B.** Correlation analysis between ROI-E_2 on day six and basal FSH. **C.** Comparison of ROI-E_2 on day six among patients of different age group. **D.** Relationship between ROI-E_2 on day six and patient age.

Good ovarian response in patients with elevated ROI-E_2 on day 6

The number of mature follicles observed on the day of oocyte pick up has shown a good association with ROI-E_2 on day 6 (Figure 2A). The patients were grouped based on their ROI-E_2 on day 6 and then compared with number of mature follicle, number of oocyte retrieved and maturity of the oocyte (Table 2). The patients with ROI-E_2 less than 50 (mean 32.98) had approximately 8 follicles on the day of oocyte pick up. However, the number of mature follicles was doubled when patients had ROI-E_2 above 150 (mean 181.6) and three times higher when ROI-E_2 was above 200 (mean 331). In addition, the number and the maturity of oocytes were also significantly increased with increase in ROI-E_2 on day 6. We plotted ROI-E_2 value on day 6 and number of mature follicles (Figure 2A), number of oocytes (Figure 2B) and maturity of the oocytes (Figure 2C) to find out their significance with ROI-E_2. We observed a significant correlation with the ROI-E_2 on day 6 for all these parameters suggesting that the patients with increased ROI-E_2 expected to yield more number of mature follicles and mature oocytes when compared with patients showing lower value of ROI-E_2 on the day 6 of controlled ovarian hyperstimulation.

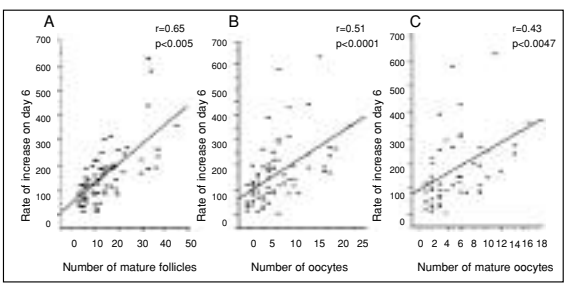

Figure 2. Relationship between ROI-E_2 on day six and **A.** Number of mature follicles aspirated; **B.** Number of oocytes retrieved; **C.** Number of mature oocytes.

ROI-E_2 and ovarian hyperstimulation syndrome

Although, elevated ROI-E_2 is suggestive of good ovarian response, the risk of developing OHSS in such patient is also an important concern. Hence the predictive value of ROI-E_2 was another important objective of this study. We analyzed the relationship between the incidence of OHSS and ROI-E_2 level on day 6 of COH. Among 113 patients, 13 had developed mild to severe forms of OHSS (11.5%) following COH. The mean ROI-E_2 in patients who did not develop OHSS was 145, and found elevated significantly ($p<0.001$) in the patients who had OHSS (Figure 3).

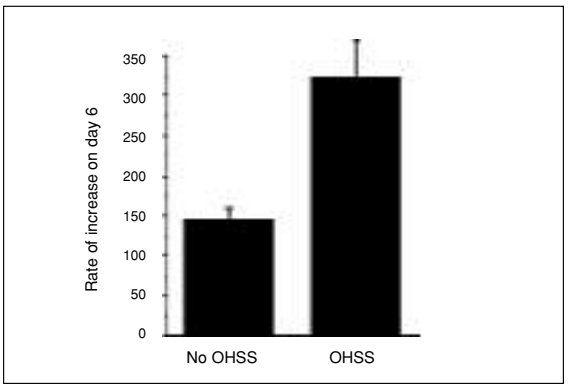

Figure 3. Comparison of ROI-E_2 in OHSS and non-OHSS patients ($p<0.001$).

Discussion

With the extensive financial burden associated with IVF cycles, it is the innate responsibility of the fertility clinic to identify prognostic factors to assist patients in making decisions regarding IVF treatment. Although, estradiol levels remain an integral part of monitoring in most ART programs, the prognostic significance of serum E_2 alone or in conjunction with ultrasound findings may be enhanced by using additional markers. In this study we made an attempt to evaluate the rate of increase in serum E_2 in response to gonadotropin stimulation and our study demonstrates for first time that ROI-E_2 on day six of COH has a predictive value in ART cycles.

The supra physiological level of E_2 produced during superovulated cycles may be influenced by both intra and extra ovarian factors. It has been reported that low peak E_2 levels are often associated with advanced maternal age, reduced number of oocytes retrieved and decreased fertilization and embryo cleavage rate (8,15). We studied the relationship between ROI-E_2 and patient age and found that patients below 35 years had increased ROI-E_2, good number of follicles and oocytes where as, a fall in ROI-E_2 observed in the patients above 36 years and the overall ovarian response in these patients was poor. This is in agreement with a previous study where patients experiencing falling E_2 exhibit many features of poor responders and a sharp decline in E_2 level during superovulation showed very poor outcome (6).

The outcome of IVF is influenced by the basal FSH levels (16). It has been reported that, high basal E_2 level (day 3), particularly in association with high basal FSH level predicts poor ovarian response to stimulation (11,17). In contrast, our study clearly demonstrated a significant negative correlation between basal FSH and ROI-E_2 on day six. Furthermore, overall ovarian response was diminished in patients with low ROI-E_2 and increased basal FSH. Hence, ROI-E_2 on day six in combination with basal FSH level may suggest the clinician to readjust the gonadotrophin dose which may possibly enhance the ovarian response at later stages of folliculogenesis.

Several studies based on E_2 estimation during COH were unable to justify the significance of E_2 in predicting ovarian response. It has been shown that low levels of serum E_2 levels after 5 days of COH are associated with poor outcome (12). In contrast, a recent study has shown no correlation between percentage decrease in E_2 level and IVF outcome (6). We observed a significant decline in the number of mature follicles, number of oocytes and their maturity among the patients showing low ROI-E_2. The poor ovarian response with low ROI-E_2 observed in this study could be explained by the direct effect of E_2 on modulating follicular microenvironment and oocyte function (18). It may also result from lack of an adequate estrogenic intrafollicular milieu, which is important for the oocyte's acquisition of developmental competence and cytoplasmic maturation, including activation, synthesis of the male pronucleus growth factor, and pre-implantation development (19).

Even though the E_2 level during COH considered as a predictor of ovarian hyperstimulation (20-22), its clinical value is still controversial (5,23). Delvigne et al. (24) suggested that a steep rise of E_2 during COH increases the likelihood of OHSS, but these studies did not clearly define objective clinical data. Earlier reports analyzed the significance of E_2 only at terminal phases of COH to predict OHSS. However, our study suggested the predictive value of ROI-E_2 on day six of COH which allow the clinician to manipulate the gonadotrophin dose to reduce the risk of developing OHSS. Although we observed elevated ROI-E_2 in the patients who developed OHSS, it has to be evaluated further to know whether dose adjustment during gonadotropin stimulation which usually done after day six of COH would provide a clear reduction in the occurrence of OHSS in these patients.

In IVF practice, it is important to identify poor responders and also patients at a risk of developing OHSS as early as possible to reduce the emotional and financial burden of therapy. Although this study has not provided data on the association between ROI-E_2 and pregnancy outcome, we demonstrated the significance of ROI-E_2 on day six of COH, which possibly help the clinician to optimize superovulation protocol accordingly to improve the ovarian response. In addition, this helps in counseling the patients much before oocyte aspiration about potential problems concerning stimulation response and chances of achieving success.

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