Area Under the Curve for Estradiol Levels Do Not Consistently Reflect Estradiol Levels on the Day of hCG Administration in Patients Undergoing Controlled Ovarian Hyperstimulation for IVF-ET

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Purpose: Most studies reported estradiol (E_2) levels attained on day of hCG administration when investigating effect of E_2 on IVF outcome. We studied whether a relationship exists between the area under the curve for E_2 levels (AUC- E_2) and E_2 levels on hCG day during IVF-ET.

Methods: Retrospectively, we analyzed data for 313 patients who completed one IVF-ET cycle each. Patients were sorted according to AUC- E_2 levels. Then we compared between each patient's own AUC- E_2 and the corresponding E_2 level on hCG day for the same patient.

Results: Although overall AUC- E_2 correlated positively with E_2 levels on hCG day, there was no consistent correlation between individual patients.

Conclusions: AUC- E_2 reflects more accurately the amount of E_2 produced by the follicles during controlled ovarian hyperstimulation. The absence of a uniform correlation between AUC- E_2 and E_2 on hCG day may result in different conclusions when studying outcomes of IVF treatment.

KEY WORDS: Area under the curve; controlled ovarian hyperstimulation; estradiol; IVF-ET.

INTRODUCTION

In most assisted reproduction programs, gonadotropins are used alone or in combination with other drugs to stimulate the growth and maturation of multiple oocytes, a process called controlled ovarian hyperstimulation (COH). This is essential because of the need to recruit a sufficient number of follicles to allow retrieval of an adequate number

of oocytes. This could improve the chance of fertilization and allow an increased number of embryos for transfer. However, the development of multiple ovarian follicles during COH is associated with the ovarian output of supraphysiologic estradiol (E_2) levels.

Recent advances in the understanding of ovarian stimulation, the techniques of oocyte retrieval, the handling of gametes, the methods of assisted fertilization, and improved conditions of culture media have steadily increased the fertilization rate. However, there has not been a corresponding increase in implantation rates, which have remained steady at 10–15% for a long time (1). Governed by multiple interactive events, embryo implantation depends mainly on the quality of embryos and the status of uterine receptivity. Several developments in

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controlled ovarian hyperstimulation (COH), fertilization, and embryo culture techniques have led to an optimization in the number and quality of embryos available for ET. In contrast, uterine receptivity has failed to benefit from parallel improvements, and this may be an important cause of the suboptimal embryo implantation rates observed in IVF-ET.

The supraphysiologic E_2 levels attained during COH may play a role in explaining the reduced implantation rate after IVF-ET treatment. However, the influence of high levels of E_2 during embryonic implantation is still controversial. Some investigators reported that COH used for IVF inhibits embryo implantation in humans (1–7) and mice (6,8) and found that high E_2 levels are associated with decreased outcome. However, other investigators found that high E_2 levels are not detrimental to IVF outcome (9–11) or the two events do not correlate (12–14).

Most of those studies reported the E_2 levels attained on the day of hCG administration rather than the area under the curve for E_2 levels attained during COH, which is expected to reflect more accurately the amount of ovarian E_2 produced during COH. In this study, we looked at the relationship between the area under the curve for E_2 production (AUC- E_2) and E_2 levels on the day of hCG administration during COH in patients undergoing IVF-ET treatment. Furthermore, we studied whether applying the two different methodologies (AUC- E_2 and E_2 levels on the day of hCG) in defining pattern of response to COH, would lead to similar or different conclusions regarding the effect of E_2 levels on the outcome of IVF-ET treatment.

MATERIALS AND METHODS

After obtaining approval from the local Institutional Research Board, data were retrospectively analyzed from the charts of patients who underwent IVF-ET cycles, one cycle each.

Study Design and Place

Data were collected retrospectively at the Infertility and In Vitro Fertilization Associates of Western New York, an academic tertiary referral center affiliated with the Department of Gynecology and Obstetrics, University at Buffalo, State University of New York (SUNY at Buffalo).

Included Data

Data were obtained from charts of the patients who underwent IVF-ET treatment during the pe-

riod from January 2001 and January 2003. We included patients who received COH with long protocol GnRH agonist down-regulation, and had E_2 levels checked at least every other day from the first day of gonadotropin stimulation until the day of hCG administration. We included patients who had their E_2 levels assayed at the same laboratory applying immunoassay methods that had comparable intra and interassays coefficient factors. We found 313 patients who met the above mentioned admission criteria.

Analysis of Data

The area under the curve for E_2 levels (AUC- E_2) attained during COH was calculated for each individual patient as well as the corresponding E_2 level on the day of hCG administration for the same patient. The AUC- E_2 was calculated by calculating the area under the curve drawn by plotting the daily E_2 levels assayed during the controlled ovarian hyperstimulation starting on the first day of gonadotropin administration until the day of hCG administration. Daily E_2 values were plotted into an Excel sheet that was created with 1-day interval between each two successive E_2 values. An arbitrary straight line was drawn between each two successive E_2 levels on every two successive days. As shown in Fig. 1: E_2 , the area under the curve, was calculated by adding the sum of the surface areas for each triangle and the corresponding rectangle in each stimulation day.

In analyzing the data, we looked at the overall correlation between AUC- E_2 and the corresponding E_2 level on hCG day for each individual patient. Then we selected two groups of patients, one representing patients who achieved pregnancy and the second group, representing the patients who did not achieve pregnancy. In each group, patients were sorted according to the AUC- E_2 in an ascending manner. Then 10 patients were selected on either side of the median level of AUC- E_2 for the whole group looking at the relationship between each two successive AUC- E_2 levels with the corresponding E_2 levels on the day of hCG stimulation for the same patient.

To study the effect of E_2 levels attained during COH on the outcome of IVF-ET treatment (achievement of clinical pregnancy as defined by the documentation of fetal cardiac activity on transvaginal ultrasound done 2 weeks after a positive pregnancy test), we sorted the patients into three groups according to the E_2 levels. First group included the patients who had the lowest 10% E_2 levels (low

Fig. 1. Shows how area under the curve for E₂ levels was calculated. Each surface area for every day of controlled ovarian hyperstimulation was calculated by adding together both surface areas for the rectangle and triangle as shown in the figure. The AUC for E₂ levels was calculated by adding the sum of each stimulation day surface area.

responders). Second group included patients who had the highest 10% of E₂ levels (high responders). Third group included the rest of the patients (80%) who had the average E₂ levels between the low and high responders (average responders).

We applied each methodology (AUC-E₂ and E₂ level on the day of hCG stimulation) in grouping patients into low, high, and average responders. We compared clinical pregnancy rates among the three different groups when sorted according to AUC-E₂ and when sorted according to E₂ on the day of hCG administration separately.

Statistical Analysis

The Student's *t* test and analysis of variance (ANOVA) were used where appropriate to compare between the E₂ levels between each two successive patients and among the selected patients, as well as correlation between AUC-E₂ and E₂ on the day of hCG administration. *P* < 0.05 was considered statistically significant. The statistical tests were performed with SigmaStat for Windows Version 1.0 software (SigmaStat Software HighEdit Professional Copyright® 1993, MicroHelp Inc and HeilerSoftware GmbH, San Rafael, CA, USA).

RESULTS

Table I shows the mean, median, and standard deviation values for AUC-E₂ levels and E₂ levels on the day of hCG administration for all patients included in the study.

Figure 2 shows the correlation between AUC-E₂ and E₂ levels on the day of hCG administration for all patients included in the study presented as corresponding values of AUC-E₂ and E₂ on hCG day for each individual patient. Values were sorted in ascending manner according to the AUC-E₂. Although the overall AUC-E₂ levels positively correlated with the E₂ levels attained on the day of hCG administration (*p* < .07), there was no uniform correlation between successive individual patients. The lack of a consistently uniform relationship between successive

Table I. The Mean, Median and Standard Deviation Values for AUC-E₂ and E₂ Levels on the Day of hCG Administration for All Patients Included in the Study

	AUC-E ₂ (pg/mL/cycle)	E ₂ on day of hCG administration (pg/mL)
Mean	9357	2399
Median	9068	2301
Standard deviation	4555	1091

Fig. 2. Shows the correlation between the AUC- E_2 and E_2 level on the day of hCG administration for all patients. There is a weak positive correlation between the two levels ($p < 0.07$). To allow both the AUC and the E_2 curves to be shown on this figure, the AUC units are in ng/dL/cycle and the E_2 levels are in pg/mL.

individual patients is further closely demonstrated in Figs. 3 and 4. Figures 3 and 4 show representative E_2 levels in two samples (pregnant and nonpregnant cycles), each consisting of 21 patients selected around the median levels of AUC- E_2 . To minimize the chance for selection bias, in Fig. 5 a representative sample of 40 cycles was randomly selected showing similar finding of lack of consistent uniform relationship between successive individual patients.

Figure 6 shows the clinical pregnancy rates among the three patient groups when sorted according to their response to COH based on the levels of E_2 levels (low, high, and average responders). When the patients were sorted according to the AUC- E_2 levels, high responders group were found to have a statistically significantly lower clinical pregnancy rate when compared to average responders ($p < 0.05$). However, when patients were sorted according to the E_2 levels attained on the day of hCG administration, there was no statistically significant difference between the high responders and average responders. On the other hand, low responders had statistically significantly lower clinical pregnancy rates when compared to average responders. This was true when

patients were sorted according to AUC- E_2 or according to E_2 levels on the day of hCG administration.

DISCUSSION

The results of this study demonstrate that although the overall AUC- E_2 positively correlated with the E_2 levels attained on the day of hCG administration, there was no consistently uniform correlation between successive individual patients. We believe that this could explain, at least in part, for the controversy in the literature among reports on the effect of E_2 levels attained during COH on the outcome of IVF-ET treatment.

When we sorted our patient population according to their response to COH applying the two different methods of assessing E_2 levels attained during COH (AUC- E_2 and E_2 levels on the day of hCG administration), we obtained different results concerning clinical pregnancy rate in high responders. High responders with high E_2 levels were associated with significantly lower clinical pregnancy rates when we looked at E_2 levels as AUC- E_2 . On the other hand, there was no statistically significant difference when

Fig. 3. Shows AUC-E₂ versus E₂ levels on the day of hCG administration for a representative sample of 21 patients who did not achieve pregnancy, selected around the mean of AUC-E₂. To allow both the AUC and the E₂ curves to be shown in this figure, the AUC units are in ng/dL/cycle and the E₂ levels are in pg/mL.

Fig. 4. Shows AUC-E₂ versus E₂ levels on the day of hCG administration for a representative sample of 21 patients who achieved pregnancy, selected around the mean of AUC-E₂. To allow both the AUC and the E₂ curves to be shown on this figure, the AUC units are in ng/dL/cycle and the E₂ levels are in pg/mL.

Fig. 5. Shows AUC-E₂ versus E₂ levels on the day of hCG administration for a representative sample of 40 patients who were randomly selected from the all treatment cycles. To allow both the AUC and the E₂ curves to be shown on this figure, the AUC units are in ng/dL/cycle and the E₂ levels are in pg/mL.

Fig. 6. Shows the clinical pregnancy rates among different patient groups when sorted according to their response to COH based on the levels of E₂ levels. Patients were sorted into low (lowest 10%), high responders (highest 10%), and average responders (middle 80%) according to E₂ levels. E₂ levels were determined according to AUC for E₂ and according to E₂ on hCG day separately.

we looked at E_2 levels as the E_2 levels on the day of hCG administration. However, there was a trend toward a lower pregnancy rate although statistically insignificant. On the other hand, low responders were associated with statistically significantly lower pregnancy rates when compared to average responders. This finding was consistent with both methods of assessing E_2 levels. It is obvious that there is a consensus in the literature on the poor outcome of IVF-ET treatment in low responders when compared to average responders (15–17).

It is clear that calculating AUC- E_2 attained during COH takes into consideration the duration of COH stimulation as well as the value of several E_2 levels rather than considering only one value for E_2 levels on the day of hCG administration. Taking into account those two variables would make AUC- E_2 more accurate methodology in studying the effect of E_2 on the treatment outcome.

As shown in Figs. 2–4, patients may have similar E_2 levels on the day of hCG administration but different AUC- E_2 . As explained above, this can be due to a difference in the duration of COH. Moreover, patients may have similar E_2 levels on the day of hCG administration and equal stimulation days but different patterns of E_2 rise along the stimulation days. This would lead to unequal levels of AUC- E_2 .

CONCLUSIONS

We believe that when studying the effect of E_2 levels attained during COH on the outcome of IVF-ET treatment, calculating AUC- E_2 might be a more reliable and accurate method in elucidating a possible effect of high E_2 levels on the treatment outcome. Applying different methodologies in defining pattern of response to COH may explain at least in part the controversy in the literature regarding the outcome of IVF-ET in high responders.

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