Estradiol production during controlled ovarian hyperstimulation correlates with treatment outcome in women undergoing in vitro fertilization–embryo transfer

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Objective: To study the value of E_2 production during controlled ovarian hyperstimulation (COH) in predicting IVF-ET outcome.
Design: Historical cohort.
Setting: Academic infertility center.
Patient(s): A cohort of 270 patients who completed 324 consecutive IVF-ET treatment cycles.
Intervention(s): None.
Main Outcome Measure(s): Area under the curve for E_2 levels (AUC-E_2) from the first day of COH until the day of hCG administration was calculated and cycles grouped into low, average, and high AUC-E_2 groups. Clinical pregnancy rates per cycle were compared among the three groups, and correlations with AUC-E_2 values were calculated for all patients and after sub-grouping according to age, COH protocol and infertility diagnosis.
Result(s): Cycles with low and high AUC-E_2 values had significantly lower pregnancy rates particularly in patients 35 years or older. There was a positive correlation between AUC-E_2 and pregnancy rates up to a certain AUC-E_2 level above which a negative correlation was found. The turning point between positive and negative correlations occurred at a significantly lower AUC-E_2 level in patients 35 years or older.
Conclusions: Estradiol production during COH correlates with IVF-ET outcome. Women >35 years of age seem more vulnerable to high E_2 levels. (Fertil Steril® 2006;86:588–96. ©2006 by American Society for Reproductive Medicine.)

Key Words: Area under the curve, controlled ovarian hyperstimulation, estradiol, IVF-ET

Since the birth of Louise Brown in 1978, IVF-ET has become the therapeutic mainstay for female infertility, with rapid expansion of IVF clinics worldwide resulting in >1% of children being conceived by some form of assisted reproduction (1). In most IVF-ET cycles, gonadotropins are used alone or in combination 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 greater number of follicles, which allows retrieval of several oocytes. This would improve the chance of fertilization and allow an increased number of embryos for transfer to give acceptable success rates. It is clear that supraphysiologic levels of E_2 are inevitably attained during COH owing to the development of multiple ovarian follicles, each contributing significantly to E_2 production which can reach levels up to 10 times or more those found during spontaneous cycles (2, 3).

The effect of such supraphysiologic E_2 levels on the outcome of IVF-ET has been the subject of intense debate with conflicting evidence (4, 5). Some investigators have shown that supraphysiologic levels of E_2 have a detrimental influence on endometrial receptivity and IVF outcome (6–16). However, others did not find high E_2 levels to be detrimental to IVF outcome (17–25).

Most of the studies observed E_2 concentrations attained on the day of hCG administration rather than considering E_2 levels along the whole period of ovarian stimulation. Calculating the area under the curve for E_2 levels (AUC-E_2) along the several days of COH is expected to reflect more accurately the amount of E_2 produced, because it takes into consideration both the duration of ovarian stimulation and several E_2 levels rather than a single level on the day of hCG administration. Recently, we reported that although the overall AUC-E_2 correlated with E_2 concentrations attained on the day of hCG administration, there was no uniform correlation between successive individual patients, and that different

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conclusions can be obtained when studying E_2 levels attained during COH by looking at E_2 levels on the day of hCG administration rather than AUC-E_2 (26). The present study looks at the correlation between E_2 levels attained during COH calculated as AUC-E_2 and the outcome of IVF-ET treatment as achievement of clinical pregnancy.

METHODS

After obtaining approval from the State University of New York at Buffalo's Institutional Review Board, we retrospectively analyzed data from charts of patients who underwent completed IVF-ET cycles and had a fresh ET.

The study was conducted in conjunction with the Infertility and In Vitro Fertilization Associates of Western New York, an academic tertiary referral IVF-ET center affiliated with the Department of Gynecology-Obstetrics, State University of New York, Buffalo.

Data were obtained from charts of the patients who underwent IVF-ET treatment during the period from January 2001 to July 2002. The study included patients who received COH and had E_2 levels checked at least every other day from the first day of COH 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 similar intraassay and interassay coefficient factors.

We found 270 patients, who completed 324 IVF-ET cycles, who met the admission criteria. Stimulation was performed with a starting dose of 150–225 IU recombinant FSH or a combination with highly purified FSH. The starting dose was decided based on the patient's clinical profile, including age, body mass index, and response in a prior gonadotropin stimulation cycle. The dose was adjusted to reach an optimum number of three follicles of ≥18 mm present on ultrasound; at that time, final oocyte maturation was achieved by administration of 10,000 IU hCG. Pituitary down-regulation was done as previously described according to the long GnRH agonist (27) or microdose (28) protocols. Owing to the retrospective nature of this study, we could not obtain embryo quality data that was valuable enough for comparison between the different groups. This was mainly due to the use of different embryo scoring systems.

Analysis of Data

Area under the curve for E_2 levels was calculated for each IVF-ET treatment cycle. The AUC-E_2 was calculated from the available E_2 concentrations along the follicular phase starting on the first day of COH until the day of hCG administration. The AUC was calculated as previously described (48).

Treatment cycles were grouped into cycles with low, medium, and high AUC-E_2. The low AUC-E_2 group included cycles in which AUC-E_2 was less than the mean minus 1 SD, the high AUC-E_2 group included cycles in which AUC-E_2 was more than the mean plus 1 SD, and the medium AUC-E_2 group included cycles in which AUC-E_2 was between that of the other two groups (mean ± 1 SD). Clinical pregnancy (defined as confirmation of fetal cardiac activity with transvaginal ultrasound approximately 4–6 weeks after embryo transfer) rates per cycle were compared among the three study groups in all cycles and after subgrouping according to age (<35 and ≥35 years), protocol applied for COH (long GnRH agonist and microdose), and infertility diagnosis (tubal, male, anovulatory, unexplained, and combined [more than one factor]).

To look at the correlation between AUC-E_2 and clinical pregnancy rate per cycle, treatment cycles were grouped according to AUC-E_2 values (increments of 3,000 pg/mL per day). Correlation between AUC-E_2 and clinical pregnancy rate per cycle was calculated for all cycles and for subgroups of cycles according to age, COH protocol, and infertility diagnosis.

RESULTS

Table 1 shows the patients' characteristics, including age, duration of infertility, number of prior IVF-ET cycles, and gravidity. These characteristics were compared among the study groups (low, medium, and high AUC-E_2) for all cycles. It is interesting that there were no statistically significant differences in any of those characteristics (data not presented).

Table 2 shows the mean value of AUC-E_2 (pg/mL per day) for the three study groups (low, medium, and high AUC-E_2) for all cycles and after subgrouping according to age and stimulation protocol. Although the mean of AUC-E_2 tended to be higher in cycles for patients <35 years old and in long GnRH agonist stimulation protocol cycles, the difference was not statistically significant. The closeness of the value of the median for AUC-E_2 to the value of the mean in all subgroups indicates a normal distribution of the AUC-E_2 values around the median. Such normal distribution is seen in Figure 1, showing the percentage distribution of AUC-E_2 values among cycles for patients <35 and ≥35 years old (Fig. 1A) and long GnRH agonist and microdose stimulation protocols (Fig. 1B). As the figures show, the low and high

TABLE 1

Patient characteristics.

Characteristic	Mean	Median	SD	Range
Age (y)	35.1	34	3.9	20–44
Duration of infertility (mo)	35.8	36	25	6–184
Number of prior IVF cycles	0.86	1	0.61	0–4
Gravidity	0.85	1	1.1	0–6

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TABLE 2

The mean value of AUC-E_2 (pg/mL/day) for the three study groups (low, medium, and high AUC-E_2).

Patient group	Mean AUC-E_2 (median)	SD	Low AUC-E_2 group	Medium AUC-E_2 group	High AUC-E_2 group
All cycles	9,182 (8,850)	4,578	<4,604	4,604–13,760	>13,760
Cycles for patients <35 yrs old	9,400 (9,068)	4,755	<4,645	4,645–14,155	>14,155
Cycles for patients ≥35 yrs old	8,959 (8,586)	4,378	<4,581	4,581–13,337	>13,337
Long GnRH agonist protocol cycles	9,608 (9,293)	4,932	<4,676	4,676–14,540	>14,540
Microdose protocol cycles	8,863 (8,379)	4,265	<4,597	4,597–13,128	>13,128

Note: There were no statistically significant differences between mean AUC-E_2 of cycles in women less than or 35 years or older or between long GnRH agonist or microdose protocol cycles.

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AUC-E_2 values were more or less equally distributed around the mean value (which is close to the median value) for AUC-E_2.

Cycles associated with low and high AUC-E_2 values (low and high AUC-E_2 groups) had significantly lower clinical pregnancy rates per cycle compared with cycles associated with medium AUC-E_2 (medium AUC-E_2 group). This was true for all cycles ($P<.05$) and after subgrouping according to age and COH protocol, as shown in Table 3, which presents actual number of treatment cycles, clinical pregnancy cycles, and clinical pregnancy rates per cycle. When analyzed according to the infertility diagnosis, the same pattern of higher pregnancy rates associated with the medium AUC-E_2 groups was maintained. However, subgrouping according to the various infertility diagnoses (tubal, male, unexplained, anovulatory, and combined) resulted in treatment cycles in each subgroup that were too few to achieve enough power for statistical analysis (data not shown).

Figure 2A shows the clinical pregnancy rate per cycle among the three study groups (low, medium, and high AUC-E_2) according to age. The difference in clinical pregnancy rate per cycle between the medium AUC-E_2 group and the other two groups (low and high AUC-E_2) was more significant in cycles for patients ≥35 years old ($P<.01$). Figure 2B shows the clinical pregnancy rate per cycle among the three study groups (low, medium, and high AUC-E_2) according to COH protocol. The difference was more marked between high and medium AUC-E_2 in the long GnRH agonist protocol ($P<.01$) than in the microdose protocol cycles ($P<.05$). The reverse was true regarding the clinical pregnancy rate per cycle between the low and high AUC-E_2 groups: The difference was more marked with the microdose protocol ($P<.01$) than with the long GnRH agonist protocol ($P<.05$).

Figure 3 shows the correlation between clinical pregnancy rate per cycle and AUC-E_2 in all cycles (Fig. 3A) and after subgrouping according to age (Fig. 3B) and stimulation protocol (Fig. 3C). In all three graphs, there was a significant positive correlation between clinical pregnancy rate per cycle and AUC-E_2 ($P<.05$) until a certain AUC-E_2 value above which a negative correlation ($P<.05$) was found. The value of the AUC-E_2 turning point between positive and negative correlation occurred at a significantly lower AUC-E_2 value in patients ≥35 years old ($P<.05$). However, this turning point did not seem to differ between long GnRH agonist and microdose stimulation protocol cycles.

DISCUSSION

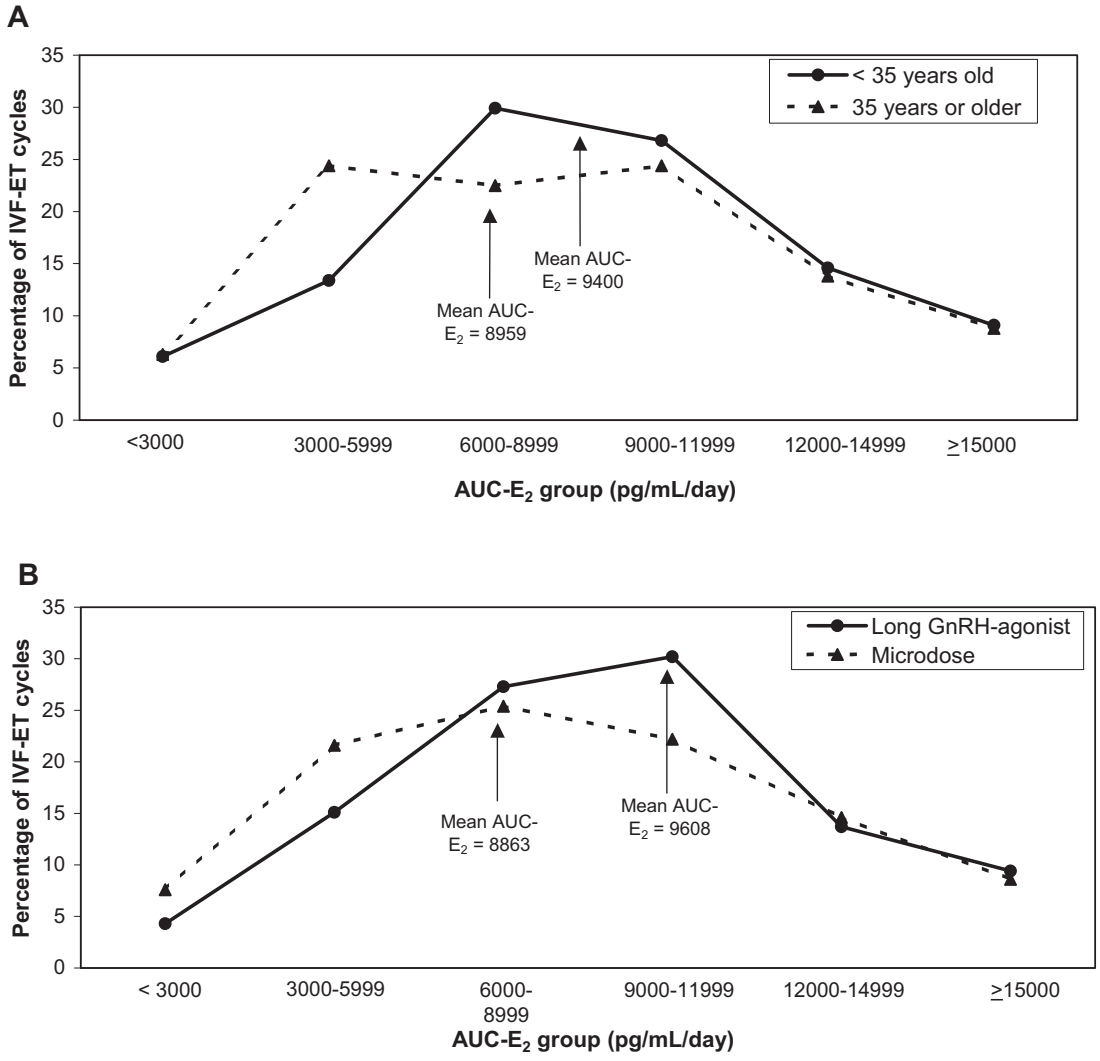
The results of the present study show that E_2 levels attained during COH have a significant correlation with the outcome of IVF-ET treatment (achievement of clinical pregnancy). Both low and high E_2 levels are associated with poor treatment outcome (low clinical pregnancy rates per cycle). Such poor outcome was independent of age, stimulation protocol, and possibly infertility diagnosis. Older women seem to be more vulnerable to the deleterious effect of high E_2 levels than younger women.

Patients who received microdose stimulation protocol had lower AUC-E_2 values compared with the long-protocol. This was due to the general practice followed in our center of reserving the microdose protocol for lower responders and for patients expected to have lower response, e.g., older women. This also explains the lower AUC-E_2 levels in women ≥35 years old, because they would have a higher chance of being in the microdose protocol as well as of being low responders.

Increasing values of AUC-E_2 were found to correlate positively with clinical pregnancy rate per cycle up to a certain point. The medium study group (mean AUC- E_2 ± 1 SD) value was 4,604–13,760 pg/mL per day, which would be the most favorable for high pregnancy rate in IVF-ET. This positive correlation was true after controlling for age, stimulation protocol, and infertility diagnosis, up to a certain level above which higher AUC-E_2 values were associated with negative outcome, i.e., lower clinical

FIGURE 1

Percentage distribution of AUC-E_2 values among cycles for patients <35 and ≥35 years old (**A**) and long GnRH agonist and microdose stimulation protocols (**B**). Most of the treatment cycles were more or less equally distributed around the mean value for AUC-E_2.



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pregnancy rate per cycle. There might be an optimum range of AUC-E_2 which is associated with the best treatment outcome, i.e., achievement of clinical pregnancy. Levels below and above such an optimum range are associated with lower pregnancy rates.

In women ≥35 years old, the negative correlation between AUC-E_2 and clinical pregnancy rate per cycle started at a lower AUC-E_2 value compared with younger women, indicating a higher vulnerability for the possible deleterious effects of high E_2 levels in the older age group. A different explanation would be that the younger patients are more resistant to the deleterious effects of the high E_2 levels. In other words, the optimum range for AUC-E_2 values is lower in older patients than in younger patients, or the optimum range is wider in younger patients.

We believe that the present study explains rather than contradicts prior studies that looked at the effect of E_2 levels attained during COH on the outcome of IVF-ET treatment. Some found high E_2 levels associated with poor IVF-ET treatment outcome (6–17) whereas others reported higher pregnancy rates in association with high E_2 levels (18–25). Two main reasons could explain such controversy: the methodology applied in investigating E_2 levels attained during COH and the clinical circumstances underlying the included patient group.

Regarding the methodology applied in previous studies, most of those studies looked at E_2 level only on one day

TABLE 3

Clinical pregnancy rates among the three study groups (low, medium, and high AUC-E_2).

Patient group	AUC-E_2 group	No. of cycles	No. of clinical pregnancy cycles	Clinical pregnancy rate per cycle
All cycles	Low	49	8	16.3%
	Medium	227	78	34.4%[a]
	High	48	10	20.8%
Cycles for patients ≤35 yrs old	Low	23	5	21.7%
	Medium	116	47	40.5%[a]
	High	25	5	20%
Cycles for patients >35 yrs old	Low	26	3	11.5%
	Medium	111	34	30.6%[a]
	High	23	2	8.7%
Long GnRH agonist cycles	Low	19	5	26.3%
	Medium	103	44	42.7%[a]
	High	17	2	11.8%
Microdose protocol cycles	Low	31	3	9.7%
	Medium	123	36	29.3%[a]
	High	31	6	19.4%

[a] Statistically significant ($P<.05$) when compared to each of the other two groups (low and high AUC-E_2 groups).

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during ovarian stimulation (day of hCG administration) or on one of the earlier days, e.g., days 3 and 5 (29), day 4 (30), or day 5 (31). In the present study, we looked at E_2 levels along the whole period of COH by calculating the AUC for those E_2 levels. This is obviously more accurate in reflecting how much E_2 the endometrium, developing oocyte, and other potential sensitive targets were exposed to. In addition, calculating AUC-E_2 value takes into consideration the duration of ovarian stimulation and timing of hCG administration, which are factors thought to affect the outcome of IVF-ET treatment.

Another important methodology factor is the cut points for the E_2 level set between high, average, and low responders, which were different among various studies. In addition, many of the previous studies failed to look at the correlation between the attained E_2 levels and the different treatment outcomes. Instead, in those studies, patients were grouped as high, average, and low responders, and pregnancy rates were compared among them. As indicated by the correlation curves found in our current study, when the cut point between high and average responders for E_2 levels is set at a higher level, lower pregnancy rates would be found with high responders, because more patients will be out of the optimum range for E_2 levels. It is clear that the opposite is true, i.e., the lower the set point for E_2 level, the higher will be the pregnancy rate with high responders, because more patients in the high responder group will be within the optimum range for E_2 levels.

Another methodology factor could be that previous studies looked at a different treatment outcome, i.e., pregnancy rate based on a positive pregnancy test instead of clinical pregnancy based on ultrasound confirmation of fetal cardiac activity. In the current study, clinical pregnancy rate per cycle was the primary outcome, to avoid the effect of early pregnancy loss, e.g., chemical pregnancies, on the results, and both comparison and correlation methodology between AUC-E_2 groups and treatment outcome were applied.

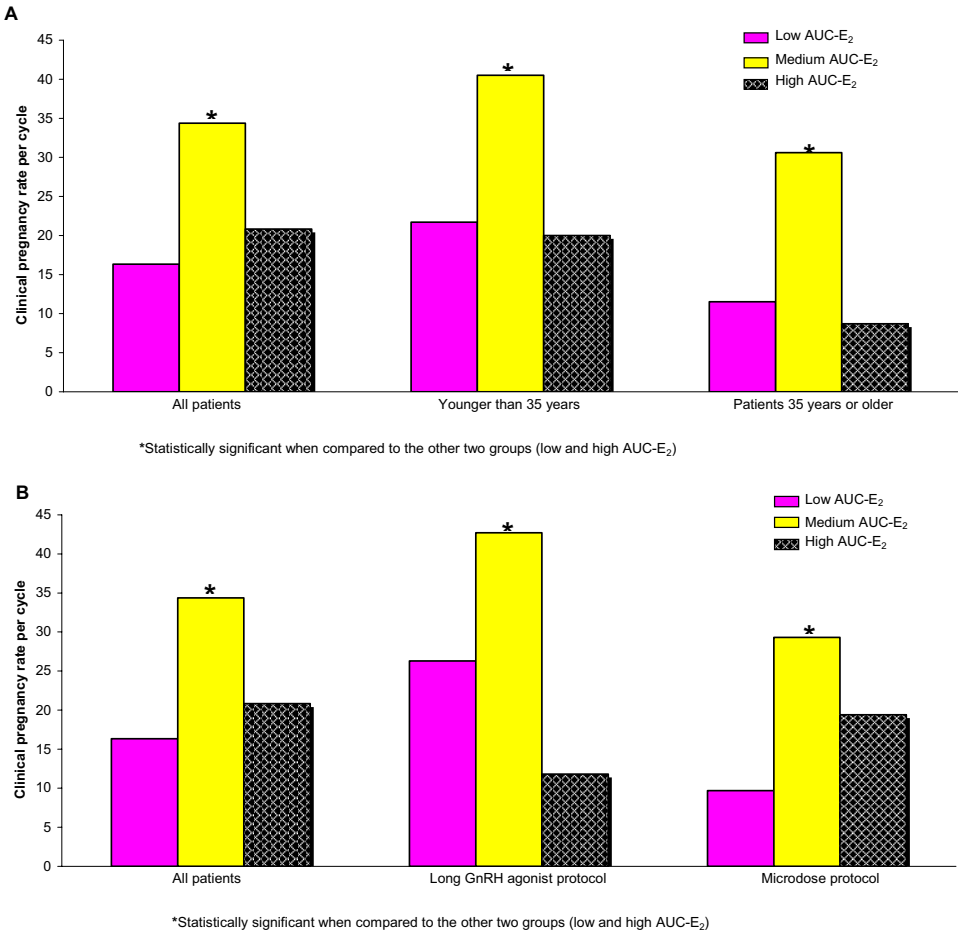
Regarding the clinical circumstances underlying IVF-ET treatment, it is clear that differences in patient populations, stimulation protocols, and attitude of the treatment physicians regarding the degree of aggressiveness during COH all would add to the explanation of the controversy and prevent defining a narrow range of E_2 values below or above which E_2 values might affect the treatment outcome.

There is both animal and human evidence for unfavorable outcome, including impaired implantation, in association with supraphysiologic E_2 levels attained during COH compared with natural pregnancy (6, 10, 12, 15, 32). In addition, many studies found higher pregnancy rates in donor oocyte recipients than patients undergoing standard IVF-ET (33–36). Although higher success rates could be attributed to better-quality oocytes from younger donors, in centers using shared oocytes, where the donor keeps half of the oocytes for herself, higher pregnancy and implantation rates were found in the recipients. Theoretically, such higher rates could be attributed to either a better endometrial environment in recipients or an adverse effect of the COH (37, 38).

Different mechanisms have been suggested to explain the adverse effect of the supraphysiologic levels of estrogen and

FIGURE 2

(**A**) Clinical pregnancy rates per cycle among the three study groups (low, medium, and high AUC-E_2) according to age. (**B**) Clinical pregnancy rate per cycle among the three study groups (low, medium, and high AUC-E_2) according to COH protocol.



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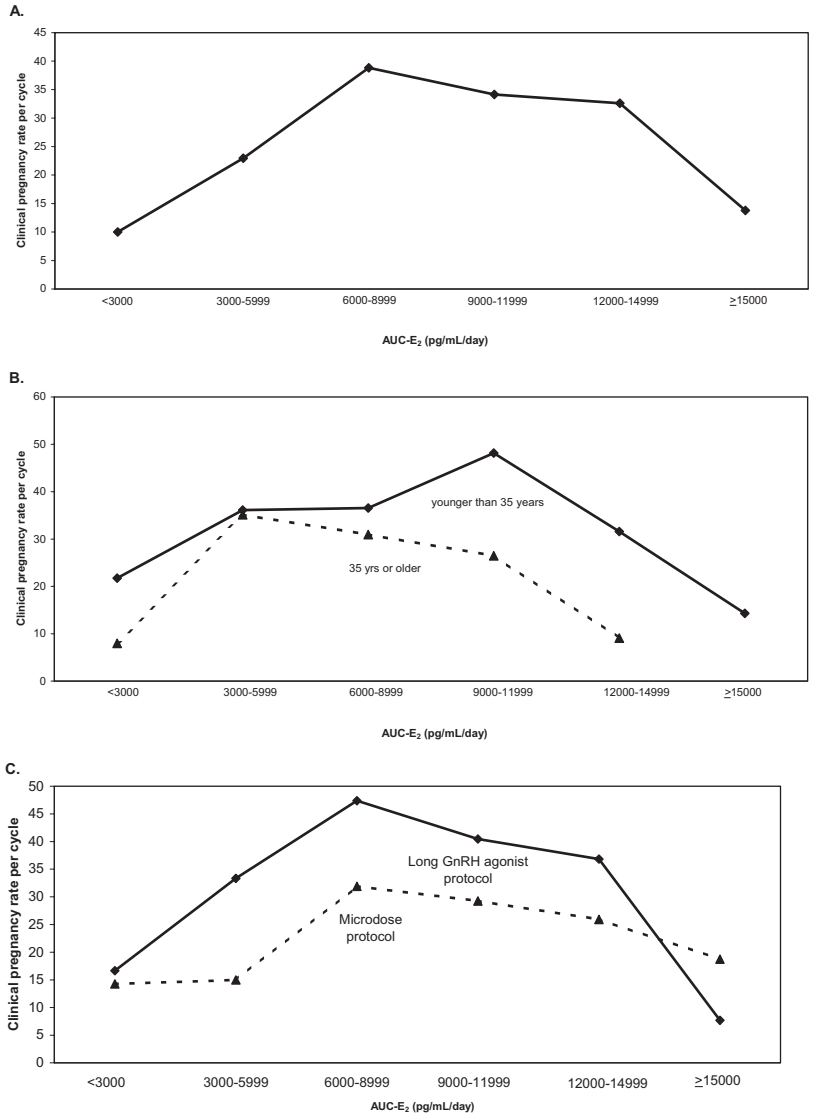
are mainly focused on possible deleterious effects on the endometrium and/or on the embryo including accelerated endometrial development (39). Valbuena et al. (40) have suggested that high E_2 levels are detrimental to endometrial receptivity and have suggested a step-down regimen to increase endometrial receptivity in high responders (16). Although the exact mechanisms have not yet been determined, it appears that excessive E_2 production during COH leads to insufficient secretory transformation of the endometrium and discordant glandular and stromal development at a time that coincides with the period of maximum uterine receptivity (41, 42). In addition, there are possible adverse effects directly on the embryo that could reduce the chance for blastocyst adhesion and implantation (43).

It is clear that the day of hCG administration would have an effect on the outcome of IVF treatment, because it would affect the AUC-E_2 by changing the number of stimulation days. Delaying hCG administration is expected to increase AUC-E_2 values by adding more stimulation days, whereas early administration of hCG would result in the opposite, i.e., lower AUC-E_2 values. There is a dilemma regarding the effect of hCG administration day on the outcome of IVF treatment, similar to the dilemma of the effect of estrogen levels on IVF outcome. In a randomized trial, Tan et al. (27) found no significant differences in pregnancy rates among three groups of patients randomized to receive hCG on different days. Group 1 received hCG when the mean diameter of the leading follicle reached 18 mm and at least two other follicles were 14 mm in diameter, and groups 2 and 3 received hCG 1 day later and 2 days later, respectively. On the other hand, other investigators found the day of hCG administration to affect the outcome of IVF treatment.

In a retrospective study, a beneficial effect of delaying hCG administration for 24 hours in IVF patients down-regulated with GnRH agonists was reported (44). This was

FIGURE 3

Correlation between clinical pregnancy rate per cycle and AUC-E_2 in all cycles (**A**) and after subgrouping according to age (**B**) and stimulation protocol (**C**).



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confirmed by a randomized controlled trial in patients undergoing IVF after using the same down-regulation with GnRH agonists (45). Other investigators reported the reverse, i.e., higher pregnancy rates when hCG was administered earlier. In a randomized controlled trial of patients undergoing IVF treated with a short GnRH agonist protocol, delaying hCG administration for 24 hours significantly decreased the chance of achieving an ongoing pregnancy (46). More recently, in another randomized trial, prolongation of the follicular phase in patients down-regulated with GnRH antagonists for IVF was associated with a significantly lower ongoing pregnancy rate without affecting oocyte or embryo quality (47).

The findings of our current study may explain, in part, such discrepancies. As mentioned earlier, the turning point between positive and negative correlation between AUC-E_2 values and clinical pregnancy rates explains the diversity of outcomes associated with varying the day of hCG administration. Early or delayed hCG administration is expected to shift the AUC-E_2 values around the turning point between negative and positive correlation phases. Pregnancy rates will be higher or lower depending on the extent and direction of shift of AUC-E_2 values around the turning point. If the shift is not marked, i.e., still within the optimum AUC-E_2 values, no effect on pregnancy rate would be expected.

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 values may be more helpful than spot E_2 levels in predicting treatment outcome. In summary, the present study shows that both low and high E_2 values attained during COH are associated with poor treatment outcome during IVF-ET cycles. Women with average response to stimulation can be expected to have an average outcome in terms of pregnancy. It seems that low E_2 values are associated with poor outcome, particularly when these values reflect low response to COH indicating poor ovarian reserve rather than when E_2 values are low because of less aggressive COH. High E_2 values seem to be associated with poor outcome only when exceeding a certain limit.

Optimizing AUC-E_2 values during COH may help improve the treatment outcome after IVF-ET. Low E_2 levels due to poor response to ovarian stimulation seem to be unavoidable in some patients, whereas the deleterious effects of very high E_2 values could be avoided by reducing E_2 values by applying less aggressive stimulation protocols or using other possible agents such as aromatase inhibitors, which carry the potential of improving IVF-ET outcome by reducing supraphysiologic E_2 levels without affecting the number of mature follicles and therefore retrieved oocytes (48–54). It is important to mention here that association does not allow us to infer causation. So, we cannot ascertain that manipulating E_2 levels would necessarily lead to significant change in the treatment outcome. Further studies are needed to define the optimum E_2 range associated with best IVF-ET outcome and the value of applying aromatase inhibitors for adjusting E_2 levels attained during COH into such optimum range.

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