Effect of the trends of estradiol level on the outcome of in vitro fertilization-embryo transfer with antagonist regimens: a single center retrospective cohort study

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Research

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Abstract

Background

The outcome of in vitro fertilization-embryo transfer is often determined according to follicles and estradiol levels following gonadotropin stimulation. However, there is no accurate indicator to predict pregnancy outcome, and it has not been determined how to choose subsequent drugs and dosage based on the ovarian response. This study aimed to make timely adjustments to follow-up medication to improve clinical outcomes based on the potential value of estradiol growth rate.

Methods

Serum estradiol levels were measured on the day of gonadotrophin treatment (Gn0), four days later (Gn4), seven days later (Gn7), and on the trigger day (HCG). The ratio was used to determine the increase in estradiol levels. According to the ratio of estradiol increase, the patients were divided into four groups: group A1 ($Gn4/Gn0 \leq 6.44$), group A2 ($6.44 < Gn4/Gn0 \leq 10.62$), group A3 ($10.62 < Gn4/Gn0 \leq 21.33$), and group A4 ($Gn4/Gn0 > 21.33$); group B1 ($Gn7/Gn4 \leq 2.39$), group B2 ($2.39 < Gn7/Gn4 \leq 3.03$), group B3 ($3.03 < Gn7/Gn4 \leq 3.84$), and group B4 ($Gn7/Gn4 > 3.84$). We analyzed and compared the relationship between data in each group and pregnancy outcome.

Results

In the statistical analysis, the estradiol levels of Gn4 ($P = 0.029$, $P = 0.042$), Gn7 ($P < 0.001$, $P = 0.001$), and HCG ($P < 0.001$, $P = 0.002$), as well as the ratios of Gn4/Gn0 ($P = 0.004$, $P = 0.006$), Gn7/Gn4 ($P = 0.001$, $P = 0.002$), and HCG/Gn0 ($P < 0.001$, $P < 0.001$) both had clinical guiding significance, and the lower one significantly reduced the pregnancy rate. The outcomes were positively linked to groups A ($P = 0.040$, $P = 0.041$) and B ($P = 0.015$, $P = 0.017$). The logistical regression analysis revealed that group A1 (OR = 0.440 [0.223–0.865]; $P = 0.017$, OR = 0.368 [0.169–0.804]; $P = 0.012$) and B1 (OR = 0.261 [0.126–0.541]; $P < 0.001$, OR = 0.299 [0.142–0.629]; $P = 0.001$) had opposite influence on outcomes.

Conclusion

Maintaining a serum estradiol increase ratio at least above 2.39 on Gn7/Gn4 may result in a higher pregnancy rate. When estradiol growth is not ideal, gonadotrophin dosage should be adjusted appropriately to ensure the desired outcome.

Introduction

During in vitro fertilization-embryo transfer (IVF-ET) cycles, controlled ovarian hyperstimulation (COH) treatment using exogenous gonadotropin (Gn) to stimulate the development of follicles is a critical step in ensuring the acquisition of enough mature eggs and a satisfactory pregnancy rate. In clinical practice, assisted reproductive technology (ART) outcome is often monitored according to the size and number of follicles and serum estradiol (E₂) level after Gn stimulation. However, the need for combined monitoring (using transvaginal ultrasound and serum estradiol) during ovarian stimulation is controversial. Additionally, no accurate indicator to predict pregnancy outcome has been identified to date, and it has not been determined how to choose subsequent drugs and doses based on the ovarian response. Some people argued that vaginal ultrasound alone should be considered to simplify treatment, as combined monitoring is costly, time-consuming, and inconvenient [1]. Neither E₂ on the day of hCG (human chorionic gonadotropin) administration nor other stages were linked to pregnancy rates in women undergoing ART cycles [2–5]. Additionally, E₂ levels were found to be a poor predictor of treatment success [6]. However, the evidence had a low overall quality [7]. Additionally, other researchers suggested that E₂ levels could be used to predict pregnancy outcomes in combination with FSH, age, inhibin B, and so on [8–10]. Some scholars suggested that poor ovarian response can be characterized by peak E₂ levels [11]. Phelps et al. and Kahyaoglu et al. [12, 13] explored the relationship between E₂ levels on the fourth day of Gn and IVF outcome and believed that estradiol level on the fourth day of COH cycle could predict the response of early follicles to ovarian stimulation. When the serum estradiol level on the fourth day of Gn was low, the current treatment cycle should be abandoned. Other groups concluded that low E₂ concentration after five days of Gn stimulation predicted a high cycle cancellation and lower pregnancy outcome even with similar numbers of oocytes and fertilization rates [14]. Lower E₂ levels on the sixth day of Gn were also associated with a lower pregnancy rate and the likelihood of live birth [8, 15, 16]. It was reported that an appropriate range for E₂ existed, and the higher one was not beneficial [17, 18]. Older women (>35 years) appeared to be more vulnerable to the harmful effects of high E₂ levels than younger women (≤ 35 years). Valbuena et al. [19] found that a high E₂ concentration affected embryonic adhesion. Additionally, a high E₂ concentration affected endometrium's receptivity [20–22]. However, Blazar et al. [23–25] discovered that higher E₂ levels on hCG day predicted a greater number of oocytes, and any adverse impact on endometrial can be conquered in IVF-ET. Additionally, according to percentile curves, Papageorgiou et al. [26] did not identify any deleterious effect of high E₂ levels. Super physiological estradiol levels also did not affect oocyte and embryo quality [27].

Although everyone has different opinions on how to determine E₂ in COH cycle, it is undeniable that determining E₂ during follicular phase has become a part of routine clinical practice over the last decade. Thus, this study aimed to determine if serum E₂ levels and E₂ growth rate during Gn ovarian stimulation were correlated with IVF and pregnancy outcomes in 335 patients on antagonist regimens. Additionally, this study aimed to determine the link between E₂ levels in different Gn stimulation periods and the IVF-ET outcomes; the number of embryos was investigated to distinguish E₂ effects on endometrium and embryos. If the hypothesis is substantiated, it may be the time to seek a new parameter to evaluate the ovarian response during Gn stimulation, adjust the dose, and ensure treatment outcome of COH.

Materials And Methods

Study subjects and protocol

From April 2017 to July 2020, our center conducted a retrospective analysis of infertility patients who underwent completed IVF-ET cycles and had a fresh ET. The institutional human ethics committee approved the study protocol.

Inclusion criteria: 1) age $\leq$ 38 years, 2) basal follicle stimulating hormone (bFSH) $\leq$ 10 IU/L, and basal antral follicle counting (bAFC) $>$ 5.

Exclusion criteria: 1) patients with chromosomal abnormalities, reproductive malformation, adenomyosis, and a history of recurrent spontaneous abortion, 2) patients undergoing coasting to prevent ovarian hyperstimulation syndrome, and 3) patients who underwent freeze-all strategy.

Ovarian stimulation protocol

Patients received IVF-ET treatment according to Fixed GnRH-ant (Cetrotide, Merck, Lyon, France) protocol [28]. On the second day of the menstrual cycle, recombinant human follicle-stimulating hormone 150-225 U (Gonal-F, Merck, Lyon, France; Puregon, MSD, Boulogne, France) was injected as Gn. Additionally, Gn doses were determined based on the patient's age, body mass index (BMI), bFSH, and bAFC. Oocytes were then collected by follicular aspiration under ultrasound, 34–36 h after triggering with GnRH-a (Triptoreline, Decapeptyl, Ipsen, France) or recombinant hCG (rhCG, Ovitrelle, Merck, Lyon, France). Eighteen hours after fertilization, embryo development was monitored daily and graded based on the number and size of blastomeres, fragmentation rate, multinucleation, and early densification. Notably, on the third day following oocyte retrieval, embryo with at least seven blastomeres (grades one and two) was defined as high quality [29].

On day 3, one or two embryos in the best shape were selected and transferred using a soft Wallace catheter. For luteal support in advance, we used an injection of progesterone (20 mg/branch, Zhejiang Xianju Pharmaceutical Co., Ltd.), 40 mg daily, and oral dydrogesterone tablets (10 mg/tablet, Abbott Healthcare Products B.V.), 20 mg per day, or progesterone vaginal sustained-release gel (90 mg/dose, Crinone VR 8%, Merck, Sherano, Switzerland), one dose daily. In addition, two bags of Chinese medicine Gushen Antai pills were utilized daily.

Measurement of serum E2

Venous blood samples were collected on the day of Gn (Gn 0), on four days of Gn (Gn4), on seven days of Gn (Gn7), and on the day of hCG (HCG). In addition, the ratio between them was calculated: Gn4/Gn0, Gn7/Gn4, HCG/Gn0, HCG/Gn4, and HCG/Gn7 represent the ratios of serum estradiol levels on Gn4 to Gn0, Gn7 to Gn4, HCG to Gn0, HCG to Gn4, and HCG to Gn7, respectively.

Pregnancy outcomes

Clinical pregnancy is defined as an intrauterine gestational sac with fetal heartbeat detected by transvaginal ultrasonography after six weeks of gestation.

The primary outcome was live birth, which was defined as the birth of at least one child with breathing and heartbeat, regardless of gestation duration.

Statistical analysis

Statistical analysis was conducted using SPSS version 26.0 (SPSS Inc., Chicago, USA). Shapiro–Wilk test was employed to assess data normality. Due to skewed distributions, quantitative variables were expressed as median (interquartile range, range between the 25th and 75th percentiles), and Mann–Whitney U and Kruskal–Wallis tests were performed. Qualitative variables were expressed as frequencies and analyzed using chi-square test. $P \leq 0.05$ was considered statistically significant.

Groups A and B were defined according to the 25th, 50th, and 75th percentiles of each ratio of E₂ levels.

Pearson correlations were used to determine the correlations between quantitative parameters and the increase in E₂ levels. The propensity scores were calculated using binary logistic regression analyses based on the following patients' characteristics: female age, infertility duration, body mass index (BMI), infertility factors, Gn usage time, and Gn dosage. We calculated crude odds ratios (OR) and adjusted OR with a 95% confidence interval (CI).

Results

Study population

From April 2017 to July 2020, we retrospectively analyzed 335 patients who received in vitro fertilization-embryo transfer (IVF-ET) with antagonist regimens in the Affiliated Hospital of Shandong University of Traditional Chinese Medicine. Table 1 summarizes the characteristics of the study population for positives and negatives in clinical pregnancy and live birth. Clinical pregnancy positives included 160 patients, and negatives included 175 patients. A total of 124 women had a viable live birth, whereas 211 women did not.

The positives and negatives were similar in BMI, infertility duration, Gn days, number of embryos transferred, endometrial thickness on transplantation day, and baseline hormone level.

However, patients with clinical pregnancy and live birth negatives were older than positive ones ($P = 0.001$; $P = 0.006$) and had a higher Gn dosage ($P = 0.001$; $P = 0.005$). The patients with clinical pregnancy and live birth positives were significantly higher than negatives with IVF-ET outcomes (both $P < 0.001$), such as the number of oocytes, fertilization, blastomere, and embryos.

Serum estradiol levels and ratios

Initially, Table 2 compares the outcomes based on E_2 level and ratio. Gn0 had no impact on IVF outcome ($P = 0.134$; $P = 0.122$). However, elevated E_2 levels following gonadotropin stimulation were correlated with higher clinical pregnancy and live birth (both $P < 0.05$), particularly E_2 of Gn7 ($P < 0.001$; $P = 0.001$) and HCG ($P < 0.001$; $P = 0.002$). In early follicular growth, estrogen increase rates were more statistically significant. Following gonadotropin stimulation, the higher serum estradiol ratios of Gn4/Gn0 (group A) and Gn7/Gn4 (group B) achieved more clinical pregnancies ($P = 0.004$; $P = 0.001$) and live births ($P = 0.006$; $P = 0.002$). In contrast, estrogen increases rates in late follicle growth (HCG/Gn4, HCG/Gn7) were similar across groups, without reaching statistical significance.

According to Table 2, data were classified into four groups based on the quartile of serum estradiol ratio. As displayed in Table 3, although group A exhibited no significant differences in terms of female age, BMI, and infertility time, group B indicated significant differences (both $P < 0.05$). For group A, the higher the estrogen growth rate, the shorter the Gn time required and the lower the Gn dose used (both $P < 0.001$), whereas for group B, the opposite was true (both $P < 0.005$). However, when estrogen growth rate increased, both groups A and B produced superior IVF-ET outcomes in terms of number of embryos harvested, blastomeres, embryos, clinical pregnancy positives, live birth, and so on (both $P < 0.05$). The ratio of group A increased as the ratio of group B decreased (both $P < 0.001$). When the ratio of group B was larger, the corresponding ratio of group A was smaller. When the values of the two groups were higher, E_2 levels on the hCG day were also higher (both $P < 0.001$).

The infertility factors included tubal factors (231, 68.9%), ovulation disorders (64, 19.1%), male factors (27, 8.1%), pelvic endometriosis (8, 2.4%), and other factors (5, 1.5%). Table 4 indicates that groups A and B did not correlate with varying infertility factors.

Effects of estradiol ratios of groups A and B on IVF-ET outcome

As indicated in Table 5, the logistical regression analysis revealed that both groups A1 (OR = 0.440 [0.223–0.865]; $P = 0.017$ vs. OR = 0.368 [0.169–0.804]; $P = 0.012$) and B1 (OR = 0.261 [0.126–0.541]; $P < 0.001$ vs. OR = 0.299 [0.142–0.629]; $P = 0.001$) had opposite influence on clinical pregnancy and live birth compared with groups A4 and B4, whereas groups A3 and B2 were weakly but significantly linked to pregnancy rates.

Discussion

The role of E_2 in IVF-ET is well known to the stage of the trigger day of hCG injection, and it indicates follicular maturation when estrogen level reaches 250 pg/mL, while its role before that stage remains controversial. In this study, the serum estrogen levels of Gn0, Gn4, Gn7, and HCG were measured, and the ratio between them was calculated to evaluate ovarian response, predict treatment outcome, and guide Gn dosage through the increase in estrogen level. In the statistical analysis, the estrogen levels of Gn4, Gn7, and HCG, as well as the ratios of Gn4/Gn0, Gn7/Gn4, and HCG/Gn0, all have clinical guiding significance, and the low growth level and rate significantly reduces clinical pregnancy and live birth rates. The increment coefficient of estrogen was observed at different stages of IVF-ET, and it was discovered that during Gn stimulation, the change in estrogen in the early and middle stages was also associated with pregnancy outcome, which may be linked to follicle growth mode. During the early follicular stage, a group of antral follicles is recruited and induced to develop. The collected follicle fluid includes low estrogen levels. At this moment, high levels of estrogen growth may be linked to the number of recruited follicles. As the follicle grows, follicular granulosa cells increase in number and show aromatase activity. Therefore, follicular fluid contained higher estrogen levels. At this moment, the increase in estrogen may be connected with follicular quality.

Second, no study has been conducted to explore the relationship between the growth rate of E_2 during Gn treatment and prognosis with antagonist regimen. According to Table 2, we revealed that serum E_2 ratios of groups A (Gn4/Gn0) and B (Gn7/Gn4) were statistically significant compared with pregnancy outcomes. As a result, we chose these two indicators for further analysis and grouping according to the 25th, 50th, and 75th percentiles. The Chi-square test and the construction of a binary logistics regression analysis model aimed at pregnancy outcome revealed that patients with lower serum E_2 ratios in groups A1 and B1 had lower clinical pregnancy and live birth rates, and group B had more significance. It is suggested that in clinical medication, estrogen levels can be observed after four days of Gn treatment, and medication can be adjusted when the increase in estrogen is not ideal to obtain satisfactory efficacy.

Third, we analyzed the influence factors of E_2 ratio in Gn stimulation cycle. According to statistical analysis, the increase in estrogen levels during the middle stage of Gn stimulation (Gn7/Gn4, group B) was associated with age, infertility years, and BMI, but not with the increase in estrogen levels during the early stage of Gn stimulation (Gn4/Gn0, group A). These results implied that basic characteristics of patients greatly affect the rate of estrogen increase during the stage of Gn7/Gn4 and may be a key factor impacting the quality of follicles. In the follicular growth, higher ratio of estrogen increase can result in a higher clinical pregnancies and live births, regardless of whether they are in group A or B. However, the link between groups A and B is just the opposite. The estrogen ratio of group B decreased as the ratio of group A increased. When estrogen growth is the fastest in the early stage, estrogen growth is slowest in the middle stage in the corresponding patients. An insufficient increase in estrogen in patients who recruit more follicles in the early stage could be due to an insufficient dose of Gn. When the association between group A and Gn usage was examined, it was discovered that the longer the Gn days and the higher the dose of Gn, the slower the early-stage estrogen increase. This may be related to the patient's baseline condition. Typically, patients with high BMI or more antral follicles receive a higher Gn initiation dosage, although a lower estrogen growth rate is generally obtained. On the contrary, in group B study, the longer the Gn days and the larger the required dosage of Gn, the faster estrogen growth in the middle stage. This may indicate the regularity of Gn dosage in follicular development process, and the dosage of Gn7/Gn4 is more critical at this stage, and it is also the time to increase Gn dosage in clinical setting.

Fourth, the main purpose of monitoring estrogen in IVF-ET is to assess the availability of adequate quantity and quality of mature oocytes on the trigger day. This study revealed that estrogen ratios increased during early and middle ovulation induction (Gn4/Gn0, Gn7/Gn4), as well as estrogen levels on hCG day (HCG), are significant for IVF-ET outcome. However, the estrogen growth ratio was not significantly different in the late stages of Gn-stimulated follicular growth (HCG/Gn4 and HCG/Gn7). Tan et al. [30] discovered no statistically significant differences in pregnancy rates among three groups of patients who

respectively received hCG on the day of the leading follicle reaching 18 mm, on the second day, and the third day. At the moment of follicular maturation, estrogen concentration may be more important than estrogen growth ratio, necessitating a rethink of the role of estrogen in trigger day selection.

Predicting outcomes in ART may allow for early treatment strategies earlier and protect patients from unnecessary physical and financial burdens throughout the treatment cycle. It is currently monitored by recurrent transvaginal ultrasonography or serum E₂. We considered that ultrasound could measure follicles growth, whereas serum E₂ levels mainly reflect follicle function. As a result, estradiol plays a critical role, despite its relatively low predictive value as a single factor. Additional parameters are required to identify more sensitive biochemical markers that may predict the probability of achieving a clinical pregnancy before hCG administration. Our study demonstrated that accurate monitoring of E₂ ratios was also a key aspect that supports the prognosis of IVF-ET outcome and dose adjustment. This provides the clinic with a new, simple, and convenient prediction method. By observing and calculating the range of E₂ increase ratio, we can guide Gn dosage, predict the likelihood of pregnancy, and evaluate cycle cancellation.

However, because elevated serum estrogen levels may lead to a higher cancellation rate of fresh transfer, this study excluded patients who did not enter fresh embryo transfer and underwent frozen embryo transfer. This is the shortcoming of this study, and the inclusion of cumulative pregnancy rates strengthens a new research avenue for this study.

Abbreviations

hCG
human chorionic gonadotropin
IVF-ET
in vitro fertilization-embryo transfer
Gn
gonadotropin
ART
assisted reproductive technology
E₂
estradiol
BMI
body mass index
Gn days
Gonadotropin days
Gn dosage
Gonadotropin dosage
Gn0
serum estradiol on the day of gonadotrophin
Gn4
serum estradiol on the four day after gonadotropin stimulation
Gn7
serum estradiol on the seven day after gonadotropin stimulation
HCG
serum estradiol on the trigger day of human chorionic gonadotropin injection
Gn4/Gn0
the ratio of serum estradiol levels on Gn4 to Gn0
Gn7/Gn4
the ratio of serum estradiol levels on Gn7 to Gn4
HCG/Gn0
the ratio of serum estradiol levels on HCG to Gn0
HCG/Gn4
the ratio of serum estradiol levels on HCG to Gn4
HCG/Gn7
the ratio of serum estradiol levels on HCG to Gn7.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Reproductive Ethics Committees of the Affiliated Hospital of Shandong University of TCM (ref approval no. SDTCM20210720). All participants provided written informed consent.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Jian-Wei Zhang played a role in the conception, design and review of the manuscript. Chun-Xiao Wei played a role in the conception and design, analysis and interpretation of the data and drafting of the manuscript. Liang Zhang and Ying-Hua Qi played a role in interpretation of the data and drafting of the manuscript, Cong-Hui Pang played a role in the analysis and interpretation of the data. All authors read and approved the final manuscript.

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Tables

Table 1: Baseline characteristics for clinical pregnancy and live birth

Note: Values are given as median (range).

Abbreviations: BMI, body mass index; Gn days, Gonadotropin days; Gn dosage, Gonadotropin dosage.

Table 2: The level and the ratio of serum E₂ for clinical pregnancy and live birth

Parameter	Clinical pregnancy positive	Clinical pregnancy negative	P-Value	Live birth positive	Live birth negative	P-Value
Number of cycles	160	175		124	211	
Age, years	31 (29, 33)	33 (30, 36)	0.001*	31 (29, 33)	32 (30, 36)	0.006*
BMI, kg/m ²	23.3 (20.7, 26.2)	23.3 (20.7,26.8)	0.880	22.5 (20.5, 26.3)	23.4(20.8,26.5)	0.347
Duration of infertility, years	3(2, 5)	3(2, 5)	0.651	3(2, 4.5)	3(2, 5)	0.580
Gn days	10(9, 11)	10(9, 11)	0.622	10(9, 11)	9(9, 11)	0.773
Gn dosage, IU	2025(1594, 2437)	2250 (1800,2968)	0.001*	20250(1575, 2437)	2212(1725,2875)	0.005*
Number of embryo transfers	2 (1, 3)	2 (1, 2)	0.146	2 (1, 3)	2 (1, 2)	0.158
Baseline FSH, IU/L	7.29(6.30, 8.67)	7.66 (6.26, 10)	0.102	7.49(6.20, 8.66)	7.40 (6.32, 9.89)	0.522
Baseline LH, IU/L	4.88 (3.78, 7.70)	4.58(3.44, 5.71)	0.246	5.00 (3.53, 7.05)	4.73(3.50, 6.54)	0.316
Baseline E ₂ , pg/mL	40.00(30.00, 55.00)	45.00(32.00, 59.00)	0.134	40.00(30.00, 54.50)	44.00(31.50, 59.00)	0.122
endometrial thickness, mm	11.05 (9.80, 12.58)	11.00 (9.82, 12.50)	0.929	11.20 (9.68, 12.93)	11.00 (9.98, 12.50)	0.570
Number of oocytes	12(9,20)	9(5, 14)	< 0.001*	13(9,19.5)	10(6, 14)	< 0.001*
Number of fertilization	7 (4, 11)	5 (2, 9)	< 0.001*	7 (4, 11)	5 (2, 9)	< 0.001*
Number of blastomere	6 (4, 10)	4 (2, 8)	< 0.001*	6 (4, 10)	5 (2, 8)	< 0.001*
Number of embryos	4 (3, 6)	2(1, 4)	< 0.001*	4(3, 6)	2(2, 4)	< 0.001*
Number of high-quality embryos	1(0, 2)	0 (0, 1)	< 0.001*	1(0, 3)	0 (0, 1)	< 0.001*
	Clinical pregnancy positive	Clinical pregnancy negative	P-Value	Live birth positive	Live birth negative	P-Value
E ₂ (Gn0), pg/mL	40(30, 55)	45(32, 59)	0.134	40(30, 54.5)	44(31.5, 59)	0.122
E ₂ (Gn4), pg/mL	572.00(308, 1067.5)	447(246.5, 782)	0.029*	594 (321,1025)	448(246.5, 6796.5)	0.042*
E ₂ (Gn7), pg/mL	1889(1089,3452)	1315(734.5, 2038)	< 0.001*	1889(1093,3280)	1432(785.5, 2281)	0.001*
E ₂ (HCG), pg/mL	4139(2243.5, 5010)	2720(1552, 4775)	< 0.001*	4166(2243,4996)	2796(1646, 4800)	0.002*
E ₂ ratio of Gn4/Gn0	12.2(7.38, 28.97)	9.88(5.41, 17.52)	0.004*	12.93(7.44, 28.92)	9.96(5.59, 18.9)	0.006*
E ₂ ratio of Gn7/Gn4	3.14(2.6, 3.99)	2.83(2.14, 3.73)	0.001*	3.24(2.63, 4.05)	2.87(2.27, 3.75)	0.002*
E ₂ ratio of HCG/Gn0	86.38(47.05, 129.7)	58.76(31.93, 100.6)	< 0.001*	87.22(47.48, 131.48)	59.73(34.47, 102.12)	< 0.001*
E ₂ ratio of HCG/Gn4	5.43(3.3, 8.29)	4.93(3.17, 8.63)	0.514	5.64(3.32,8.39)	4.99(3.22, 8.44)	0.562
E ₂ ratio of HCG/Gn7	1.57(1.15, 2.33)	1.70(1.14, 2.64)	0.113	1.62(1.18, 2.39)	1.66(1.13, 2.54)	0.245

Note: Values are given as median (range).

Abbreviations: E₂, estradiol; Gn0, serum estradiol on the day of gonadotrophin; Gn4, serum estradiol on the four day after gonadotropin stimulation; Gn7, serum estradiol on the seven day after gonadotropin stimulation; HCG, serum estradiol on the trigger day of human chorionic gonadotropin injection; Gn4/Gn0: the ratio of serum estradiol levels on Gn4 to Gn0; Gn7/Gn4: the ratio of serum estradiol levels on Gn7 to Gn4; HCG/Gn0: the ratio of serum estradiol levels on HCG to Gn0; HCG/Gn4: the ratio of serum estradiol levels on HCG to Gn4; HCG/Gn7: the ratio of serum estradiol levels on HCG to Gn7.

Table 3: Parameters and outcome of IVF-ET as a function of serum E₂ increase

	A1	A2	A3	A4	P-Value	B1	B2
Number of cycles	84	84	84	83		84	84
Age, years	32(29,34)	31.5(29,34)	31(30,35)	33(30,35)	0.61	33(30,36)	32(30,35)
BMI, kg/m ²	22.60(20.70,26.20)	23.05(20.40,26.40)	23.40(20.40,27.00)	24.00(21.70,27.30)	0.41	25.40(22.00,29.05)	22.80(21.35,25.85)
Duration of infertility, years	3(2,4)	3(1,5)	3(2,5)	3(1,5)	0.59	4(2,6)	3(2,4.5)
Gn days	11(10,12)	10(9,11)	9(8,10)	9(8,10)	< 0.001*	9(8,10)	9(9,11)
Gn dosage, IU	2700(2250,3300)	2250(1725,2625)	2025(1725,2400)	1770(1500,2100)	< 0.001*	1900(1500,2400)	2175(1812,2700)
endometrial thickness, mm	10.50(9.30, 12.30)	11.05(9.55, 12.90)	11.00(9.65, 12.70)	11.50(10.50, 12.50)	0.102	11.00(9.80, 12.40)	10.90(10.00, 12.30)
Number of eggs	8(4,12)	10(6,15)	10(8,16)	16(12,23)	< 0.001*	9(5,14.5)	11(6,16)
Number of fertilization	4(2,7)	6(3,9)	6.5(4,11)	8(6,13)	< 0.001*	5(2,8)	6(3,9)
Number of blastomere	3(2,6)	5(3,8)	6(4,10)	7(5,12)	< 0.001*	4(2,7)	5(3,8.5)
Number of embryos	2(1,4)	4(2,6)	4(2,6)	5(3,8)	< 0.001*	3(2,5)	3(2,5.5)
Number of high-quality embryos	0(0,1)	1(0,2)	1(0,2)	1.5(0,3)	< 0.001*	1(0,2)	1(0,1)
Clinical pregnancy	32/84(38.1%) ^a	39/84(46.4%)	48/84(57.2%)	47/83(56.6%)	0.040* ^a	31/84(36.9%) ^c	44/84(52.4%)
Live birth	24/84(28.6%) ^b	29/84(34.5%)	40/84(47.6%)	37/83(44.6%)	0.041* ^b	21/84(25.0%) ^d	35/84(41.7%)
E ₂ ratio of Gn4/Gn0	–	–	–	–		15.28(7.36,32.33)	12.85(7.85,25.94)
E ₂ ratio of Gn7/Gn4	3.28(2.70,4.04)	3.15(2.32,4.18)	3.12(2.55,4.08)	2.70(2.08,3.15)	< 0.001*	–	–
E ₂ (HCG), pg/mL	2166(1148,3616)	2592(1589,4800)	3176(2197,4800)	4870(3904,5010)	< 0.001*	2251(1277,4800)	3212(1895,4870)

Note: Values are given as number (percentage) or median (range).

Group A1 (ratio of Gn4 to Gn0≤6.44), group A2 (6.44<ratio of Gn4 to Gn0≤10.62), group A3 (10.62<ratio of Gn4 to Gn0≤21.33), group A4 (ratio of Gn4 to Gn0>21.33); group B1 (ratio of Gn7 to Gn4≤2.39), group B2 (2.39<ratio of Gn7 to Gn4≤3.03), group B3 (3.03<ratio of Gn7 to Gn4≤3.84), group B4 (ratio of Gn7 to Gn4>3.84).

a. In the pairwise comparison of group A in clinical pregnancy, the comparison between group A1 and group A3 was statistically significant (P=0.013), and group A1 and group A4 was statistically significant(P =0.016).

b. In the pairwise comparison of group A in live birth, the comparison between group A1 and group A3 was statistically significant (P=0.011), and group A1 and group A4 was statistically significant(P =0.032).

c. In the pairwise comparison of group B in clinical pregnancy, the comparison between group B1 and group B2 was statistically significant (P =0.044), and group B1 and group B4 was statistically significant(P =0.002).

d. In the pairwise comparison of group B in live birth, the comparison between group B1 and group B2 was statistically significant (P =0.022), and group B1 and group B3 was statistically significant(P =0.033),and group B1 and group B4 was statistically significant(P =0.002).

Table 4: Relationship between serum E₂ increase and Infertility factors

	A				χ^2	P	B				χ^2	P
	A1	A2	A3	A4			B1	B2	B3	B4		
Tubal factors	60	66	54	51	16.788	0.158	56	59	64	52	10.625	0.561
Ovulation disorders	13	13	18	20			16	17	9	22		
Male factors	9	5	7	6			7	5	7	8		
Pelvic endometriosis	1	0	3	1			2	1	2	0		
Other factors	1	0	2	5			3	2	2	1		

Note: χ^2 -test.

Table5. Binary logistic regression on clinical pregnancy and live birth with the ratio of serum E₂.

95% CI					95% CI				
Dependent variable: clinical pregnancy	P-Value	OR	Lower	Upper	Dependent variable: live birth	P-Value	OR	Lower	Upper
Independent variables					Independent variables				
A	0.073				A	0.076			
A1	0.017	0.440	0.223	0.865	A1	0.012	0.368	0.169	0.804
A2	0.197	0.639	0.324	1.261	A2	0.281	0.689	0.350	1.357
A3	0.030	0.434	0.204	0.923	A3	0.080	0.547	0.278	1.074
B	0.001				B	0.004			
B1	0.000	0.261	0.126	0.541	B1	0.001	0.299	0.142	0.629
B2	0.045	0.504	0.259	0.984	B2	0.038	0.485	0.245	0.960
B3	0.584	0.834	0.435	1.598	B3	0.820	0.928	0.486	1.769

Note: The independent variables also included Gn dosage, duration of infertility, BMI, female age and cause of infertility. We defined the group A4 and B4 as the last reference category.

Abbreviations: OR, odds ratio; CI, confidence interval;