Only extremely high peak serum estradiol adversely affects endometrial receptivity in IVF treatment

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Abstract

Purpose To explore the effect of different concentrations of peak serum estradiol levels on endometrial receptivity quantitatively. Methods In our reproductive medicine center, two best quality of day 3 (D3) embryos were transferred or frozen according to E 2 and progesterone levels on the day of human chorionic gonadotropin (hCG) administration and the number of oocytes retrieved. The remaining embryos were cultured to blastocyst stage and frozen. The patients were then categorized into three groups. The patients with frozen-thawed D3 embryo transfer in artificial cycles without blastocyst frozen served as group 1, those with fresh D3 embryo transfer without blastocyst frozen as group 2, and those with fresh D3 embryo transfer with blastocyst frozen as group 3. Each group was further stratified into 4 sub-groups according to E 2 levels on the day of hCG administration. Clinical pregnancy rate, implantation rate and abortion rate of frozen-thawed and fresh D3 embryo transfer were compared among the three groups in the same stratified E 2 levels. Results For E 2 <7,000 pg/mL, group 1 and group 2 had similar clinical pregnancy rate and implantation rate. But for E 2 ≥7,000 pg/mL, the clinical pregnancy rate in group 1 was significantly higher than in group 2 (p<0.05). For E 2 <7,000 pg/mL, pregnancy rate and implantation rate in group 1 were significantly lower than those in group 3 (P<0.05). But for E 2 ≥7,000 pg/mL, the pregnancy rate in group 1 was significantly higher than in group 3 (P<0.05). There was no significant difference in the abortion rate between group 1 and group 2, or between group 1 and group 3. Conclusions High serum E 2 concentration does not impair implantation and pregnancy rates unless exceeding a certain limit (e.g. 7,000 pg/mL) on the day of hCG administration. Since peak E 2 level was related to OHSS and adverse pregnancy outcomes, further study is needed to set a threshold peak E 2 level for fresh embryo transfer.

Introduction

As a key part of assisted reproductive technology (ART), in an effort to recruit more follicles, controlled ovarian hyperstimulation (COH) leads to supraphysiological levels of estradiol (E 2). However, the impact of E 2 on endometrial receptivity is not fully understood. As seen in some basic medical investigations the E 2 and Progesterone (P) following COH with exogenous gonadotropins may impair the endometrium, such as functional genomic delay of the endometrium [1] and gene expression profile disturbance [2,3]. There is a dynamic shift from cyclic proliferation to a secretory morphology during the window of implantation orchestrated directly or indirectly by E 2 and P. High levels of E 2 may contribute to a failure in the transition after COH, which is likely to affect clinical outcomes of IVF [4].

However, the results reported from existing clinical studies are controversial. Some investigators have found a negative association between the probability of pregnancy and E 2 concentrations on the day of hCG administration [5–9]. While other studies have shown that high E 2 levels do not impair pregnancy rates [10–20]. For example, a total of 2,921 infertile women were grouped according to high P and high E 2, leading to the conclusion that elevated P was detrimental to the IVF pregnancy outcome, but high
serum \( E_2 \) concentrations was not [15]. Even using the same method, comparing birth outcomes between roughly stratified \( E_2 \) levels, investigators come to a different conclusion [5,9,12].

The reasons for the different conclusions are as follows: Firstly, most of the studies mentioned above ignored the adverse effect of \( P \) elevation on the day of \( hCG \) administration, a frequent event in IVF/ICSI cycles, on clinical pregnancy rate (CR) [21]. Another reason is that baseline data were not matched in some studies when exploring the correlation between \( E_2 \) and the achievement of pregnancy [9,20,22]. At last the small sample size of some studies might contribute to different conclusion [5,7,19,23].

To explore the effect of different concentrations of peak serum estradiol levels on endometrial receptivity quantitatively and acquired dose-effect relationship, in this study, patients with elevated \( P \) levels (\( P > 1.5 \) ng/mL) on the day of \( hCG \) administration were excluded and baseline data of different groups were matched. In a retrospective study with more than 6,000 cycles included, clinical outcome between day 3 (D3) fresh embryo transfer at different concentrations of high peak serum \( E_2 \) levels (4 groups and \( E_2 \) increased by 2000pg/ml gradually) and frozen-thawed D3 embryo transfer in artificial cycles was compared for the first cycle. Our data showed that only extremely high concentrations of \( E_2 \) (\( \geq 7,000 \) pg/mL) were detrimental to the endometrium receptivity.

**Materials And Methods**

All IVF/ICSI cycles with gonadotropin-releasing hormone (GnRH) agonist protocol between December 2016 and December 2018 were retrospectively included and only the first stimulation cycle was considered. This was a retrospective study of routinely collected clinical data.

GnRH agonist (Diphereline; Beaufour Ipsen, France) was given from mid-luteal phase for about 14 days. Then the rFSH (Puregon Pen, N. V. Organon, the Netherlands) or rhFSH (Gonal-F; Serono, Switzerland) ranged from 75–300IU per day was injected for about 10 days. The dose was based on patient age, ovarian reserve testing and weight. When at least 3 follicles reached 17mm in diameter hCG 10000 IU (HCG, Livzon Pharmaceutical Group Inc., China) was used to trigger ovulation. Transvaginal oocyte retrieval was performed 34–36 h later after hCG administration. Fertilization was achieved using standard IVF or ICSI.

In our reproductive medicine center, two best quality of D3 embryos were transferred or frozen and the remaining embryos were cultured until blastocyst stage and then frozen. If \( P \) levels on the day of \( hCG \) administration <1.5 ng/mL, the number of oocytes retrieved <20 and \( E_2 < 9,000 \) pg/mL, two best quality of embryos were transferred on day 3. The luteal phase was supported with progesterone. If \( P \) levels on the day of \( hCG \) administration were >1.5 ng/mL or the number of oocytes retrieved >20 or \( E_2 > 9,000 \) pg/mL, two best quality of D3 embryos were frozen and thawed, which were transferred in hormonally controlled cycles. That is, estradiol valerate (Shire Pharmaceuticals, UK) was taken orally at 4 mg/day from day 2 to day 5, at 6 mg/day from day 6 to day 9, and at 8 mg/day from day 10 onwards and \( E_2 \) levels varied from 100 to 200 pg/ml. Provided the endometrial thickness reached 8 mm or more,
progesterone (40 mg/d) (Utrogestan, Besins Healthcare, China) was intramuscularly injected and
dydrogesterone (20mg/d) (Dydrogesterone, Abbott Healthcare Products B. V., China) was taken orally to
translate endometrium into the secretory phase. Embryos were transplanted at the fourth day of
progesterone injection.

In this study patients undergoing the transfer of two fresh or frozen-thawed D3 embryos in the first IVF-ET
cycle were categorized into three groups: patients with frozen-thawed D3 embryo transfer in hormonally
controlled cycles without blastocyst formation and frozen (group 1), patients with fresh D3 embryo
transfer without blastocyst formation and frozen (group 2), patients with fresh D3 embryo transfer with
blastocyst formation and frozen (group 3). The design of groups is shown in Figure1. In order to explore
the effect of different concentrations of peak serum E$_2$ levels on endometrial receptivity quantitatively
and acquire dose-effect relationship, each group was further stratified into four sub-groups according to
E$_2$ levels on the day of hCG administration: subgroup <3,000 pg/mL; subgroup 3,000–5,000 pg/mL;
subgroup 5,000–7,000 pg/mL; subgroup ≥7,000 pg/mL. 3,000–5,000 pg/mL is not including 5,000
pg/mL, 5,000–7,000 pg/mL is not including 7,000 pg/mL.

IVF clinical outcome of D3 embryo transfer were compared among group 1, group 2 and group 3
according to the stratified E$_2$ levels, which included clinical pregnancy rate, implantation rate and abortion
rate. Clinical pregnancy rate was identified with the presence of an intrauterine gestational sac with fetal
cardiac activity on transvaginal ultrasound two to three weeks after a positive pregnancy test.
Implantation rate reflects the number of gestational sacs divided by the number of embryos transferred.
Miscarriage rate was defined as pregnancy loss after sonographic visualization of an intrauterine
gestational sac.

The characteristics of patients included number of patients, age, duration of infertility, baseline FSH, AFC,
body-mass index, number of oocytes retrieved, metaphase II oocytes (M2) number, two visualized
pronuclei (2PN) number, endometrial thickness, gonadotropin time and gonadotropin dose. Baseline data
of different groups were matched. The one-way analysis of variance (ANOVA) test was used to evaluate
differences of data characteristics between group 1 and group 2 in the same E$_2$ levels on the day of hCG
administration, and between group 1 and group 3. Clinical outcomes between the groups was compared
using χ²-test. Significance was assumed at P < 0.05. All statistical analyses were carried out using the
Statistical Package for Social science version 22.0 (SPSS, Chicago, IL, USA) and a statistical program by
Graph Pad Prism 7 (San Diego, CA, USA).

Results

On the basis of the criteria mentioned above, a total of 6,532 patients were enrolled from 16,000 IVF/ICSI
cycles at the ART center, including 726, 2,096 and 3,710 patients in group 1, group 2 and group 3,
respectively.
Table 1 shows the clinical outcomes between group 1 and group 2 according to stratified E$_2$ levels. For subgroups <3,000 pg/mL, 3,000–5,000 pg/mL and 5,000–7,000 pg/mL, the clinical pregnancy rate and Implantation rate showed no significant difference between group 1 and group 2. But for subgroup $\geq$7,000 pg/mL, the clinical pregnancy rate in group 1 was significantly higher than in group 2 (66.7% vs. 48.5%, P = 0.039). When E$_2$ $\geq$7,000 pg/mL the implantation rate in group 1 was higher than in group 2 although the difference was not statistically significant (44.2% vs. 34.8%, P = 0.130).

Table 2 shows the clinical outcomes between group 1 and group 3 according to stratified E$_2$ levels. For subgroups subgroups <3,000 pg/mL, 3,000–5,000 pg/mL and 5,000–7,000 pg/mL, the pregnancy rate and Implantation rate in group 1 was significantly lower than in group 3 (P<0.05). But interestingly, for subgroup $\geq$7,000 pg/mL, the clinical pregnancy rate in group 1 was higher than in group 3 (66.7% vs. 50.3%, P = 0.026). But for subgroup $\geq$7,000 pg/mL, the implantation rate showed no significant difference between group 1 and group 3 (44.2% vs. 36.9%, P = 0.154).

As shown in Table 1 and 2, the abortion rates showed no significant difference between group 1 and group 2, or between group 1 and group 3 according to different E$_2$ levels.

Table 3 shows the patient characteristics of the three groups according to stratified E$_2$ levels, including number of patients, age, duration of infertility, baseline FSH, AFC, body-mass index, number of oocytes retrieved, M2 number, 2PN number, endometrial thickness, gonadotropin time and gonadotropin dose.

**Discussion**

Suitable development of the endometrium is an important determinant of successful implantation. In the follicular phase during COH, supraphysiological levels of E$_2$ levels, resulting from exogenous gonadotropin, are up to more than ten times those of spontaneous cycles [24,25]. Comparing the endometrium, after ovarian stimulation for IVF cycles with that of natural cycles, a large proportion of dysynchronous glandular and stromal differentiation occurs followed by premature secretory changes [26]. There is a significant difference of perhaps 218 and 133 genes that belong to the class of implantation genes, which may affect the basic progress of endometrial receptivity [1]. The well-regulated gene and histological structure of the human endometrium are altered due to steroidogenesis disturbance, suggesting that endometrium exposed to high E$_2$ levels for a long time during COH does not have the same receptive status as it does during the natural cycle.

But the association between supraphysiologic E$_2$ levels on the day of hCG administration and the outcome of IVF remains controversial [27]. Some studies have reported that markedly increased E$_2$ levels on the day of hCG administration had an adverse effect on implantation and pregnancy [7,23,28]. However, other studies had different results even using the same method. Data showed that higher E$_2$ levels would probably lead to a higher pregnancy rate [5,19,20,29], or, that there was no association between E$_2$ levels on the day of hCG administration and clinical pregnancy [8,10,17,18,23,30,31]. However, an analysis of 400,135 IVF cycles showed that the live birth rate (LBR) rose with an increase in the
number of eggs, up to 15, then plateaued between 15 and 20 eggs and steadily declined beyond 20 eggs. Therefore, supraphysiologic E\textsubscript{2} may not damage the endometrium [32]. However, the studies did not match the women's age, ovarian response and embryo quality among the different groups and a definite conclusion cannot be obtained as to whether different concentrations of supraphysiologic E\textsubscript{2} levels do harm to the endometrium.

A meta-analysis of over 60,000 cycles indicated that P elevation (P>1.5 ng/mL) on the day of hCG administration was a frequent event and affected nearly 20% IVF/ICSI cycles. The detrimental effect of P can be noted at levels even from 0.8 to 1.1 ng/ml in the general IVF population [33].

In order to come to a clear conclusion and get the dose-effect relationship we analyzed more than 6,000 cycles and compared clinical outcomes between fresh D3 embryo transfer in the environment where E\textsubscript{2} is gradually increasing and frozen-thawed D3 embryo transfer in the environment where E\textsubscript{2} varied from 100 to 200 pg/ml for the first time. The participants with elevated P levels (P>1.5 ng/mL) on the day of hCG administration were excluded and all baseline characteristics of the subgroups, especially the age known as the best predictor of embryo quality, were matched. Interestingly, the results of the current study did not show any difference in clinical outcomes between group 1 and group 2 when E\textsubscript{2} levels were lower than 7,000 pg/mL. The results indicate that with increasing levels of E\textsubscript{2} concentrations within 0–7,000 pg/mL, the supraphysiological levels of E\textsubscript{2} were not harmful to endometrium receptivity. Another study also supported these results. In that study, the high serum E\textsubscript{2} concentrations did not appear to alter endometrial receptivity in patients undergoing COH in IVF-ET cycles compared with recipients of donor oocytes. The implantation rate, pregnancy rate, pregnancy loss rate and delivered pregnancy rate were similar for both groups, even the higher E\textsubscript{2} levels observed in IVF-ET recipients.\textsuperscript{30} However, the author did not show a correlation between clinical outcomes and different concentration of E\textsubscript{2} levels. The average peak E\textsubscript{2} level was 3,000 pg/mL.

In this study if high peak serum E\textsubscript{2} was really harmful to endometrial receptivity D3 fresh embryo transfer in the group 2 should have worse clinical outcomes than the D3 frozen embryo transfer in the group 1. However, this expected difference was appeared only when E\textsubscript{2} ≥ 7,000 pg/mL. The patients without blastocyst frozen (groups 1 and 2) had a worse clinical outcome than the group 3 (E\textsubscript{2} <7,000 pg/mL), which indicated poor embryo quality [34]. But even in such a situation, group 1 had higher implantation rates and clinical pregnancy rates than group 3 when E\textsubscript{2} surpassed 7,000 pg/mL. The above results further proved that such an extremely high concentration of E\textsubscript{2} seriously affects endometrial receptivity. However, this study was a retrospective analysis and selective embryo frozen according to E\textsubscript{2} and P levels on the day of hCG administration and the number of oocytes retrieved may affect the results of the study. But all baseline characteristics among the subgroups, especially the age known as the best predictor of embryo quality, were matched and only two best quality of D3 embryos were transferred or frozen, which reduced bias caused by embryo quality to the most extent.
This conclusion can be supported by a cohort of 270 cycles. The research used another method by calculating area under the curve for E₂ levels (AUC-E₂ values). They showed that high E₂ values seem to have a poor outcome only when up to a certain degree [28]. Another study showed that the best chance of live birth was associated with the number of eggs, around 15, and live births trended downwards with >20 eggs. The result also suggested that only extremely high concentrations of E₂ affected embryo implantation [35].

However, such high levels of E₂ after COH are rare in clinical situations. Around 5% women will obtain such high levels of serum E₂ in our center. It remains unclear why supraphysiological levels of E₂ less than 7,000 pg/mL did not adversely affect endometrial receptivity. One hypothesis is that endometrial E₂ receptors are saturated at very low concentrations of E₂ and extra E₂ has no effect on the endometrium. For example, our data showed that endometrial thickness had no linear relationship with serum E₂. It rose with an increasing peak E₂ level and reached plateau when E₂ was at the very low level of 800–900 pg/mL (Supporting information).

Although, in most cases, supraphysiological peak E₂ level had no effect on endometrial receptivity, ovarian hyperstimulation syndrome (OHSS), one of the most serious complications during ART, was related to peak E₂ level. In our center, the rate of OHSS in patients with E₂ level ≥7,000 pg/mL is about 15%. One study showed that the combination of a threshold of 18 follicles and/or E₂ of 5,000 pg/mL yields an 83% sensitivity rate with a specificity as high as 84% for the severe OHSS case [36]. Another independent study [37] confirmed similar findings and showed that the rate of OHSS became much more clinically significant after 15 oocytes (0–5 oocytes: 0.09%; 6–10 oocytes: 0.37%; 11–15 oocytes: 0.93%; 16–20 oocytes: 1.67%; 21–25 oocytes: 3.03%). In our study if peak E₂ level was between 5000–7,000 pg/mL the number of oocytes retrieved was about 14. From this year, according to standard operating procedure in our center the threshold of peak E₂ levels for whole embryo freezing was reduced to 7,000 pg/mL. Furthermore, elevated peak E₂ may have downstream implications for aberrant implantation, placentation, and adverse pregnancy outcomes. Peak E₂ level >3,069.2 pg/mL is associated with increased odds of low birthweight (LBW) term singletons after fresh IVF-ET cycles. LBW is associated with adult cardiovascular disease, diabetes, and dyslipidemia [38].

**Conclusion**

Our results indicated that supraphysiological level of E₂ did not impair implantation and pregnancy rates unless exceeding a certain limit (e.g. 7,000 pg/mL) on the day of hCG administration in IVF cycles. But further study is needed to set a threshold peak E₂ level for fresh ET since supraphysiological peak E₂ level was related to OHSS and adverse pregnancy outcomes.

**Declarations**
Author Contributions

Conception and design: KQ; acquisition and analysis of data: JS; wrote the first draft: LeiJ; review and correction: TL, LiuJ, HS, LiJ, YL and GZ.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The study was approved by the Institutional Review Board at Huazhong University of Science and Technology, China, approval number TJ-IRB20170902. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/ or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Tables

Table 1  Clinical outcomes between group 1 and group 2
| estradiol | Group 1 | Group 2 | P   |
|----------------|---------|---------|-----|
| **Clinical pregnancy rate (%)** |         |         |     |
| <3000          | 43.2(98/227) | 43.7(516/1181) | .885 |
| 3000≤to<5000   | 45.7(127/278) | 46.5(281/604) | .816 |
| 5000≤to<7000   | 49.7(80/161)  | 48.6(119/245) | .826 |
| ≥7000          | 66.7(40/60)   | 48.5(32/66)   | .039 |
| **Implantation rate (%)** |         |         |     |
| <3000          | 25.1(114/454) | 29.0(686/2362) | .075 |
| 3000≤to<5000   | 27.9(155/556) | 30.9(373/1208) | .201 |
| 5000≤to<7000   | 32.3(104/322) | 33.5(164/490) | .728 |
| ≥7000          | 44.2(53/120)  | 34.8(46/132)  | .130 |
| **Miscarriage rate (%)** |         |         |     |
| <3000          | 15.3(15/98)   | 15.7(81/516)  | .922 |
| 3000≤to<5000   | 14.2(18/127)  | 17.1(48/281)  | .460 |
| 5000≤to<7000   | 6.3(5/80)     | 9.2(11/119)   | .446 |
| ≥7000          | 15(6/40)      | 9.4(3/32)     | .473 |

**Table 2  Clinical outcomes between group 1 and group 3**

| estradiol | Group 1 | Group 3 | P   |
|----------------|---------|---------|-----|
| **Clinical pregnancy rate (%)** |         |         |     |
| <3000          | 43.2(98/227) | 52.8(487/922) | .009 |
| 3000≤to<5000   | 45.7(127/278) | 56.7(913/1611) | .001 |
| 5000≤to<7000   | 49.7(80/161)  | 56.8(560/986)  | .092 |
| ≥7000          | 66.7(40/60)   | 50.3(96/191)   | .026 |
| **Implantation rate (%)** |         |         |     |
| <3000          | 25.1(114/454) | 37.0(683/1844) | .000 |
| 3000≤to<5000   | 27.9(155/556) | 40.7(1310/3220) | .000 |
| 5000≤to<7000   | 32.3(104/322) | 40.5(799/1972) | .005 |
| ≥7000          | 44.2(53/120)  | 36.9(141/382)  | .154 |
| **Miscarriage rate (%)** |         |         |     |
| <3000          | 15.3(15/98)   | 13.6(66/487)   | .646 |
| 3000≤to<5000   | 14.2(18/127)  | 11.7(107/913)  | .426 |
| 5000≤to<7000   | 6.3(5/80)     | 13.0(73/560)   | .083 |
| ≥7000          | 15(6/40)      | 16.7(16/96)    | .810 |

**Table 3  Comparison of baseline characteristics of patients**
|                                | E<sub>2</sub> <3000 pg/ml | E<sub>2</sub> 3000-5000 pg/ml | E<sub>2</sub> > 7000 pg/ml |
|--------------------------------|----------------------------|----------------------------|--------------------------|
| **No. of Patients**            | Group 1  | Group 2  | Group 3  | Group 1  | Group 2  | Group 3  | Group 1  | Group 2  | Group 3  | Group 1  | Group 2  | Group 3  |
| **Age (years)**                | 31.77±4.63 | 32.01±4.74 | 31.24±4.40 | 31.01±4.66 | 30.37±4.56 | 30.11±4.09 | 278      | 604      | 1611     |
| **infertility (years)**        | 4.55±3.26  | 4.84±3.203 | 4.39±3.38  | 4.22±3.55  | 4.45±3.38  | 4.06±3.07  | 181      | 245      | 986      | 60      | 66      | 194      |
| **FSH (mIU/ml)**               | 8.04±2.55  | 7.55±2.34  | 7.11±1.84  | 7.11±1.97  | 7.10±1.80  | 6.96±2.07  | 227      | 1181     | 922      | 278     | 604     | 1611     |
| **AFC**                        | 9.98±6.01<sup>b</sup> | 11.69±5.37 | 12.94±5.16 | 15.10±5.62<sup>b</sup> | 13.97±5.34 | 14.98±5.52 | 3000-5000 pg/ml | 5000-7000 pg/ml | > 7000 pg/ml |
| **BMI**                        | 21.89±2.75 | 22.47±3.13 | 22.72±3.22 | 21.46±2.87 | 21.58±2.74 | 21.89±2.95 | 27.8±7.82 | 25.12±6.74 | 26.30±5.47 |
| **No. of oocytes retrieved**   | 7.10±3.83  | 6.61±6.68  | 9.49±3.20  | 12.12±5.07<sup>a</sup> | 11.00±3.10 | 12.66±3.46 | 3000-5000 pg/ml | 5000-7000 pg/ml | > 7000 pg/ml |
| **M2**                         | 6.19±2.88<sup>b</sup> | 5.79±2.58  | 8.64±2.97  | 10.29±4.55<sup>a</sup> | 9.34±2.89  | 11.50±3.26 | 6.19±2.88<sup>b</sup> | 5.79±2.58  | 8.64±2.97  | 10.29±4.55<sup>a</sup> | 9.34±2.89  | 11.50±3.26 |
| **2PN**                        | 4.05±1.74  | 3.62±1.80  | 6.01±2.40  | 6.18±3.04<sup>a</sup> | 5.19±2.34  | 7.90±2.88  | 4.05±1.74  | 3.62±1.80  | 6.01±2.40  | 6.18±3.04<sup>a</sup> | 5.19±2.34  | 7.90±2.88  |
| **Endometrial thickness**      | 10.81±2.76 | 11.24±2.40 | 11.31±2.48 | 11.08±2.66 | 11.34±2.33 | 11.27±2.35 | 10.81±2.76 | 11.24±2.40 | 11.31±2.48 | 11.08±2.66 | 11.34±2.33 | 11.27±2.35 |
| **Gn time**                    | 9.85±1.93  | 9.80±1.87  | 9.66±1.56  | 10.09±1.50 | 9.79±1.45  | 9.69±1.30  | 9.85±1.93  | 9.80±1.87  | 9.66±1.56  | 10.09±1.50 | 9.79±1.45  | 9.69±1.30  |
| **Gn dose (IU)**               | 32.76±9.28 | 31.98±9.07 | 30.03±7.98 | 30.04±8.38<sup>b</sup> | 29.02±7.99 | 27.78±6.93 | 32.76±9.28 | 31.98±9.07 | 30.03±7.98 | 30.04±8.38<sup>b</sup> | 29.02±7.99 | 27.78±6.93 |

Note: Values expressed as mean ±SD; ANOVA.  
<sup>a</sup>P<.05 (versus group 2);  
<sup>b</sup>P<.05 (versus group 3)

**Figures**
Figure 1

The group designing in this study. Group 1 (without blastocyst frozen): frozen D3-embryo transfer but no embryos left for the future. Group 2 (Without blastocyst frozen): fresh D3-embryo transfer but no embryos left for the future. Group 3 (With blastocyst frozen): fresh D3-embryo transfer and at least one embryo frozen for the future.

Supplementary Files

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