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Impact of relative estradiol changes during ovarian stimulation on blastocyst formation and live birth in assisted reproductive technology

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This study aimed to evaluate the predictive value of relative change in E2 levels during controlled ovarian stimulation (COS) on embryo development and pregnancy outcomes in assisted reproductive technology (ART). We retrospectively analyzed 9,376 patients who underwent their first fresh ART cycle from January 1, 2020, to December 31, 2022. Patients were classified into four groups based on relative change in E2 levels: low response group, moderate response group, moderate-high response group, and high response group. The primary outcomes were blastocyst formation rate, clinical pregnancy rate, and live birth rate, while secondary outcomes included miscarriage rate and ectopic pregnancy rate. Most cycles (96.5%) demonstrated an increase in E2 levels during COS. The blastocyst formation rate significantly increased across the groups (low response group: 0.13, moderate response group: 0.21, moderate-high response group: 0.28, high response group: 0.34; $P < 0.001$). Multivariable logistic regression showed significantly higher blastocyst formation rates in the moderate response group (adjusted OR = 2.012, 95% CI: 1.687–2.399), moderate-high response group (adjusted OR = 4.613, 95% CI: 3.853–5.523), and high response group (adjusted OR = 11.295, 95% CI: 9.192–13.880) compared to the low response group. Both clinical pregnancy rate and live birth rate were significantly higher in the moderate-high response group and high response group compared to the low response group (clinical pregnancy rate: 54.5% and 61.5% vs. 35.5%, adjusted RR = 1.21 [95% CI: 1.03–1.42] and 1.27 [95% CI: 1.08–1.51]; live birth rate: 44.9% and 52.0% vs. 25.7%, adjusted RR = 1.27 [95% CI: 1.06–1.52] and 1.35 [95% CI: 1.11–1.64]). However, no significant differences were observed in either clinical pregnancy rate or live birth rate between the moderate response group and low response group (clinical pregnancy rate: adjusted RR = 1.07 [95% CI: 0.91–1.25]; live birth rate: adjusted RR = 1.11 [95% CI: 0.92–1.33]). No significant differences in miscarriage rate or ectopic pregnancy rate were observed across the groups. Higher E2 responses were associated with improved embryo development and better pregnancy outcomes.

Keywords IVF/ICSI outcomes, Estrogen, Controlled ovarian stimulation, Ovarian response, Pregnancy outcomes

Successful ART depends on the development of at least one embryo that can progress to the blastocyst stage, successfully implant in the uterus, and continue developing until a live birth. However, most human oocytes in ART cycles fail to develop into blastocysts suitable for transfer. To improve the likelihood of obtaining viable embryos, COS with exogenous gonadotropins is commonly used to promote the synchronized development of multiple follicles, yielding a higher number of mature oocytes in one cycle. Serum estradiol (E2) levels are an important component of assessing COS response, but the predictive value of changes in E2 levels for ART outcomes remains controversial. Several studies have shown that higher E2 levels during COS may be associated with better in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) success rates[1,2], while excessively high E2 levels during COS may create suboptimal conditions in the preimplantation uterine environment,

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leading to abnormal placentation[3]and increasing the risk of adverse neonatal outcomes such as small–for–gestational–age (SGA) infants[4]and low birth weight (LBW)[5]. Other researchers argue that E2 concentrations on the day of hCG administration alone are poor predictors of IVF/ICSI outcomes[6,7]and may not independently increased the risk of LBW and SGA[8].

It remains unclear whether E2 levels during COS can independently predict IVF/ICSI outcomes or if a critical threshold exists that significantly affects live birth rates. Previous studies have indicated that E2 levels are significantly correlated with the number of preovulatory follicles, retrieved oocytes, and resulting embryos[9,10], and have suggested that these levels can be used to predict follicle and embryo development during assisted reproduction. However, relatively few studies adopt arbitrary E2 thresholds for stratified analysis to predict embryo development and pregnancy outcomes[1,8,9,11,12], but these studies often yield inconsistent conclusions, limiting the reliability of E2 levels as a prognostic marker.

While E2 levels in ART has been widely studied, few investigations have examined how changes in E2 levels predict pregnancy outcomes[13]. This study explores the predictive value of relative E2 changes during COS on embryo development and ART outcomes. By investigating these relative changes, we hope to provide a more accurate assessment of E2 dynamics to optimize the prognostic value of E2 in IVF/ICSI treatment.

Materials and methods

Study design and participants

In this retrospective cohort study, we identified all women aged 21–52 years who underwent fresh assisted reproductive cycles at the Reproductive Center of Liuzhou Hospital, Guangzhou Women and Children's Medical Center, between January 1, 2020 and December 31, 2022. This study only included patients undergoing their own egg stimulation cycles, and no donor egg cycles were included. The analysis was restricted to patients who entered their first cycle with complete clinical data available (n = 9,376), representing 81.1% of the total cycles during this period. We excluded cycles with inaccurate medical records or missing data, as well as patients with polycystic ovary syndrome (PCOS) or endometriosis due to their potential to introduce heterogeneity in ovarian response and pregnancy outcomes, which could confound the interpretation of our findings (Fig. 1). This study included all fresh assisted reproductive cycles for the analysis of embryological outcomes, without specific differentiation of PGT cycles. However, for the analysis of pregnancy outcomes, only fresh transfer cycles were included, and PGT cycles, which result in cryopreservation following biopsy, were excluded.

Patients' clinical characteristics such as age and duration of infertility were self-reported. Infertility diagnosis, medication protocols, and fertilization plans were recorded by physicians. Height and weight were measured and recorded on-site prior to the appointment, from which BMI was calculated (weight kilograms divided by the square of height in meters). Cycle-specific information, including oocyte and embryo development, days of embryo transfer, and the number of embryos transferred, was documented by the reproductive laboratory.

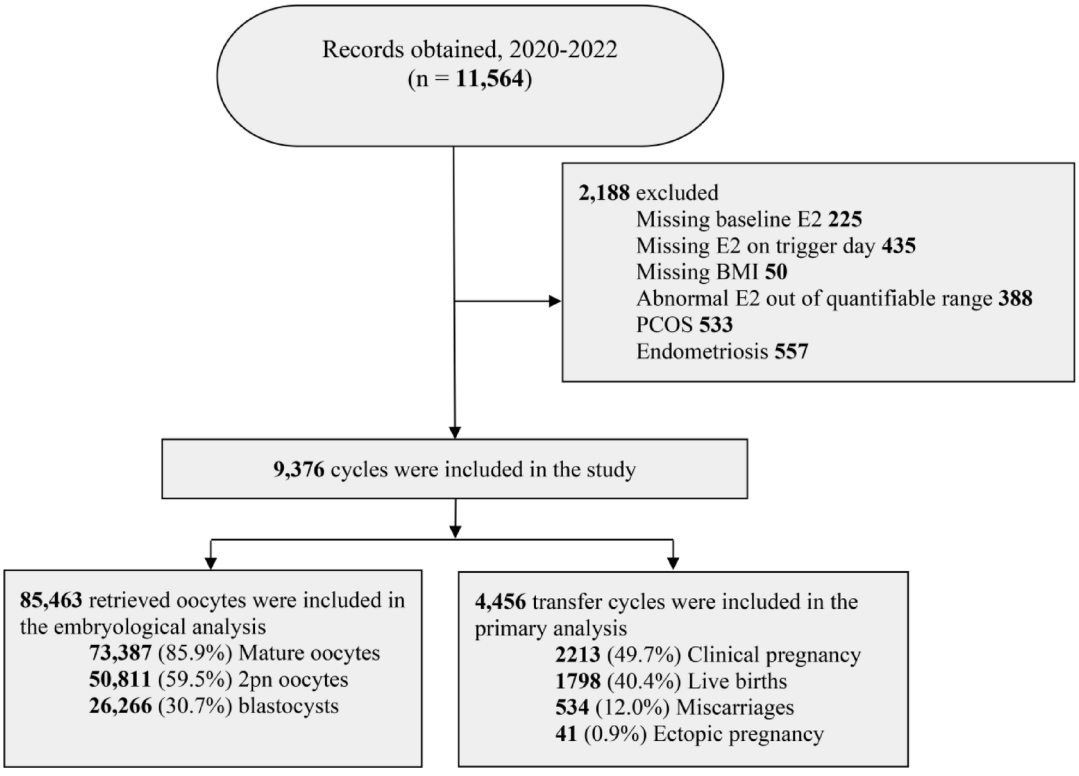

Fig. 1. Outline of the selection process, transfer cycle type, embryo stage at transfer and outcomes of embryo transfer cycles included in this study.

Only cycles with at least one embryo transfer at the cleavage or blastocyst stage were included in the clinical pregnancy analysis. Serum hormone values were obtained from the databases of the Clinical Laboratory and Genetics Laboratory.

Controlled ovarian stimulation protocols

The GnRH agonist protocol was employed for patients with good ovarian reserve (AFC ≥ 5) and normal baseline hormone levels, which were defined as FSH < 10 mIU/ml, AMH between 1.0 and 1.4 ng/ml, and AFC between 6 and 15. Downregulation began one week after ovulation using subcutaneous GnRH-a injections, such as triptorelin (0.1 mg/day or long-acting 3.75 mg) or leuprorelin (3.75 mg). Downregulation success was defined by specific hormone levels (E2 < 50 pg/ml, LH < 5 mIU/ml, FSH < 5 mIU/ml) and uterine ultrasound criteria, including an endometrial thickness < 5 mm and ovarian follicles of < 8 mm. Once downregulation was achieved, gonadotropin stimulation was initiated with doses ranging from 100 to 300 IU/day, adjusted based on age, BMI, and AFC. Follicular growth was monitored via transvaginal ultrasound and serum hormone levels every 2–3 days. Ovulation was triggered when dominant follicles reached ≥ 18 mm using HCG (5,000–10,000 IU), GnRH agonists, or a dual trigger (HCG + GnRH agonist), followed by egg retrieval 34–36 h later. Luteal support began on the day of egg retrieval, with progesterone administered vaginally, orally, or intramuscularly, and continued for 10 weeks if pregnancy was confirmed.

The GnRH antagonist protocol is suitable for patients with varying ovarian responses, including those with good, moderate, or poor ovarian reserve. Baseline assessments are performed on cycle days 2–3, including hormone levels (FSH, LH, E2, AMH) and transvaginal ultrasound to assess AFC and the presence of any ovarian cysts or abnormalities. For patients with good ovarian reserve, the classification criteria are FSH < 10 mIU/ml, AMH ≥ 1.0 ng/ml, and AFC ≥ 5. For patients with lower ovarian reserve or poor responders, the gonadotropin dose is adjusted accordingly based on baseline assessments, and for poor responders, a higher starting dose of gonadotropins (≥ 300 IU/day) may be used to stimulate follicular development effectively. Gonadotropin stimulation commenced with initial doses of 150–450 IU/day, adjusted according to patient-specific factors. A GnRH antagonist (e.g., cetrorelix 0.25 mg/day or ganirelix 0.25 mg/day) was added when the lead follicle reached 14 mm to prevent premature LH surges. Ovulation was triggered with HCG, GnRH agonists, or a dual trigger, followed by egg retrieval 34–36 h later. Luteal support was initiated on the day of egg retrieval and continued as described above.

The progestin-primed ovarian stimulation (PPOS) protocol was primarily used for patients with diminished ovarian reserve, repeated implantation failure, or specific uterine conditions requiring embryo freezing[14]. For these patients, the classification criteria include AMH ≤ 1.0 ng/ml or a history of repeated implantation failure or specific uterine conditions. Medroxyprogesterone acetate (MPA, 10 mg/day) was administered together with gonadotropins (150–300 IU/day), and follicular development was monitored via ultrasound and blood tests. Ovulation was triggered when the dominant follicle reached ≥ 18 mm, and all embryos were cryopreserved for subsequent transfer cycles.

The minimal stimulation protocol was reserved for patients with diminished ovarian reserve or previous poor response to stimulation. The classification criteria include patients with AMH < 1.0 ng/ml or a history of poor ovarian response to stimulation. This protocol is also indicated for patients with a high risk of ovarian hyperstimulation syndrome (OHSS), as it uses lower doses of gonadotropins. Oral clomiphene citrate (50–100 mg/day) or letrozole (2.5–5 mg/day) was started on cycle days 2–3, often in combination with low-dose gonadotropins (HMG 75 IU/day). Follicular growth was monitored every 2–3 days using ultrasound, and medications were adjusted accordingly. Ovulation was triggered when dominant follicles reached ≥ 18 mm, followed by egg retrieval 34–36 h later. Luteal support was provided as described in the other protocols.

Endocrine hormone measurements

Hormone levels (FSH, LH, E2, P4) were measured at multiple time points: baseline levels during the early follicular phase (cycle day 2–3 for antagonist protocols) or after achieving downregulation (E2 < 50 pg/ml for GnRH agonist protocols), on the day of HCG administration, and 12–14 days post-transfer to confirm pregnancy. These measurements were conducted using a chemiluminescent immunoassay (CLIA) on an Abbott i2000SR analyzer (Abbott Park, Illinois, USA) in a certified clinical laboratory with intra- and inter-assay coefficients of variation maintained below 10%, ensuring reliable and accurate results.

Exposures

The exposure factor is defined as the ratio of trigger day E2 to baseline E2, calculated using the formula (trigger day E2-baseline E2)/baseline E2. Based on this ratio, patients were divided into four groups: Low E2 response group (< 13.52), moderate response group (13.52–34.24), moderate-high response group (34.24–70.05), and high response group (> 70.05). As this is the first study to evaluate relative changes in E2 levels during controlled ovarian stimulation, no established thresholds exist in the literature. Therefore, we adopted a quartile-based approach to ensure objective and statistically robust groupings. Patients in the lowest quartile (< 13.52) were designated as the control group for reference.

Endometrial preparation protocol

In all fresh transfer cycles, the endometrial preparation protocol was standardized. Endometrial thickness was monitored using transvaginal ultrasound, starting from the mid-to-late follicular phase of controlled ovarian stimulation. The criterion for transfer eligibility was an endometrial thickness ≥ 7 mm on the day of ovulation trigger (HCG administration). Luteal support was initiated on the day of oocyte retrieval and included vaginal progesterone (e.g., 200 mg twice daily) or intramuscular progesterone (e.g., 40 mg daily), which was continued until 10 weeks of gestation in patients with confirmed pregnancy.

Outcomes

The primary outcomes were blastocyst formation rate, clinical pregnancy rate, and live birth rate. The blastocyst formation rate was defined as the ratio of successfully cultured blastocysts to either the number of follicle punctures or 2PN oocytes. The clinical pregnancy is defined as the presence of an intrauterine gestational sac observed via ultrasound, or records of live birth or miscarriage. The live birth is defined as the birth of one or more live infants. Secondary outcomes included oocyte retrieval rate, fertilization rate, miscarriage rate, and ectopic pregnancy rate. The number of follicle punctures refers to the total number of follicles attempted to be aspirated for oocyte retrieval. The number of oocytes retrieved refers to the total number of oocytes obtained after each puncture. The number of mature oocytes refers to the number of mature oocytes (MII stage). The number of 2PN oocytes refers to the number of normally fertilized oocytes with two pronuclei. The miscarriage rate is defined as the loss of an intrauterine pregnancy before 20 weeks of gestation. The ectopic pregnancy rate refers to the proportion of pregnancies occurring outside the uterine cavity.

Statistical methods

Given that the data did not conform to a normal distribution, baseline and cycle characteristics were presented as medians with interquartile ranges (IQR). Continuous variables, including hormone levels, were compared between groups using the Mann-Whitney U test. To control for the impact of covariates with statistically significant differences between groups, analysis of covariance (ANCOVA) was employed. Chi-square tests were used to analyzed blastocyst formation and pregnancy outcomes between groups, and relative risk (RR) with 95% confidence intervals (Cl) was calculated using log-binomial regression models. Both crude and adjusted analyses were performed to account for potential covariates that could influence pregnancy outcomes. Statistical significance was set at 0.05, and all tests were two-tailed. All statistical analyses were conducted using SPSS 26.0 and the R statistical language in the RStudio.

Ethics approval

This study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center Liuzhou Hospital (Approval No. 2024 – 211), and the committee waived the requirement for individual informed consent, as the data were de-identified and this was a retrospective analysis in compliance with relevant ethical guidelines and regulations.

Results

We analyzed a total of 9,376 fresh, first-time assisted reproductive cycles. During COS, the majority of assisted reproductive cycles showed an increase in E2 levels compared to baseline ($n = 9{,}047$, 96.5%). However, a minority of cycles experienced a decrease in E2 levels following COS ($n = 329$, 3.5%). In this study, patients were divided into four groups based on the ratio of (E2 level on the day of trigger - baseline E2 level)/baseline E2 level). The groups were classified as follows: low response (ratio < 13.25), moderate response (13.52 ≤ ratio < 34.24), moderately-high response (34.24 ≤ ratio < 70.05), and high response (ratio ≥ 70.05), with 2,344 cases in each group.

Embryological outcomes

Among these four response groups, there was a decreasing trend in patient age: 39.0 years in the low response group, 37.0 years in the moderate response group, 35.0 years in the moderate-high response group, and 33.0 years in the high response group. The differences in age between groups were statistically significant ($p < 0.05$). Although the differences were small, the BMI in the low response group was statistically different from that in the moderate-high response group and high response group ($p < 0.05$). Additionally, baseline FSH levels and LH levels on the day of trigger were significantly higher in the low response group compared to other groups, while P4 levels on the day of trigger were significantly lower than those in the higher response groups. Significant differences were also observed between groups in terms of infertility diagnosis and ovarian stimulation protocols; pelvic factor infertility was more prevalent in patients with high E2 response, while diminished ovarian reserve (DOR) was more common in patients with low E2 response (Table 1).

Figure 2; Table 2 present the outcomes of 85,463 oocytes retrieved from 93,363 follicle punctures. There was a significant weak positive correlation between the degree of E2 response and the ratio of oocytes, M2 oocytes, and 2PN oocytes retrieved per follicle puncture, and a moderate positive correlation with the blastocyst formation rate (Spearman's rho = 0.516, $p < 0.01$). Pairwise comparisons among the four E2 response groups showed significant differences in blastocyst formation rate between each group ($p < 0.001$). After adjusting for covariates based on significant differences in baseline characteristics between groups, these significant differences in blastocyst formation rate persisted ($p < 0.001$). When simplifying the E2 response into two groups, the "low E2 response group" included patients with a ratio ≤ 34.24, and the "high E2 response group" included those with a ratio > 34.24. Chi-square tests revealed a significant difference in the blastocyst formation rate per puncture between the two groups (0.2645 vs. 0.7875; $p < 0.001$).

Univariable logistic regression demonstrated that the blastocyst formation rate was significantly higher in the moderate response group (OR = 3.65, 95% CI: 3.17–4.21, $p < 0.001$), moderate-high response group (OR = 13.14, 95% CI: 11.38–15.19, $p < 0.001$), and high response group (OR = 44.75, 95% CI: 37.72–53.08, $p < 0.001$) compared to the low response group (Supplementary Table 1). As illustrated in Fig. 2, this relationship is nearly linear, indicating that as E2 response increases, the blastocyst formation rate rises progressively. Subsequently, variables found to be significant in the univariable logistic regression were included in a multivariable logistic regression model to control for potential confounders. The results showed that the blastocyst formation rate remained significantly higher in the moderate response group (adjusted OR = 1.60, 95% CI: 1.04–2.47, $p = 0.033$), moderate-high response group (adjusted OR = 3.68, 95% CI: 2.38–5.68, $p < 0.001$), and high response

Characteristics median (IQR)	Low response (*n* = 2344)	Moderate response (*n* = 2344)	Moderate-high response (*n* = 2344)	High response (*n* = 2344)
Age, y	39.0 (36.0–42.0)	37.0 (33.0–41.0)	35.0 (31.0–38.0)	33.0 (30.0–36.0)
Age group, No. (%)				
< 30	83 (3.5)	201 (8.6)	380 (16.2)	577 (24.6)
30–33	264 (11.3)	407 (17.4)	609 (26.0)	741 (31.6)
34–37	503 (21.5)	640 (27.3)	667 (28.5)	636 (27.1)
38–41	731 (31.2)	653 (27.9)	481 (20.5)	327 (14.0)
> =42	763 (32.6)	443 (18.9)	207 (8.8)	63 (2.7)
Infertility duration	4.0 (2.0–8.0)	4.0 (2.0–7.0)	4.0 (2.0–7.0)	3.0 (2.0–6.0)
BMI	22.2 (20.5–24.4)	22.2 (20.3–24.4)	21.9 (20.0–24.2)	21.6 (19.7–23.7)
BMI group, No, (%)				
< 20	428 (18.3)	470 (20.1)	526 (22.4)	626 (26.7)
20–24.9	1418 (60.5)	1381 (58.9)	1359 (58.0)	1331 (56.8)
25–29.9	426 (18.2)	447 (19.1)	406 (17.3)	347 (14.8)
> =30	59 (2.5)	38 (1.6)	46 (2.0)	31 (1.3)
Basal FSH	7.2 (5.2–10.2)	6.3 (5.1–8.2)	5.6 (4.7–6.7)	5.2 (4.4–6.0)
Basal LH	3.0 (2.1–4.4)	3.0 (2.2–4.1)	2.9 (2.2–4.0)	3.2 (2.4–4.2)
Basal P4	0.2 (0.1–0.4)	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.2 (0.2–0.3)
FSH on trigger Day	12.3 (9.4–15.8)	14.1 (11.0–17.3)	14.2 (11.2–17.2)	12.9 (9.8–16.3)
LH on trigger Day	2.7 (1.4–5.0)	1.5 (0.9–2.9)	1.0 (0.7–1.9)	1.0 (0.7–1.8)
P4 on trigger Day	0.2 (0.2–0.3)	0.3 (0.2–0.4)	0.4 (0.3–0.6)	0.6 (0.4–0.8)
Fertilization method, No. (%)[b]				
IVF	1308 (55.8)	1338 (57.1)	1431 (61.0)	1415 (60.4)
ICSI	1036 (44.2)	1006 (42.9)	913 (39.0)	929 (39.6)
Infertility diagnosis, No. (%)[c]				
Diminished ovarian reserve	1538 (65.6)	826 (35.2)	247 (10.5)	46 (2.0)
Male factor	218 (9.3)	249 (10.6)	298 (12.7)	303 (12.9)
Tubal factor	132 (5.6)	271 (11.6)	400 (17.1)	372 (15.9)
Recurrent pregnancy failure	89 (3.8)	134 (5.7)	156 (6.7)	172 (7.3)
Pelvic inflammatory disease	307 (13.1)	635 (27.1)	954 (40.7)	1111 (47.4)
Uterine factor	112 (4.8)	143 (6.1)	156 (6.7)	167 (7.1)
Others	133 (5.7)	273 (11.6)	386 (16.5)	448 (19.1)
Ovarian stimulation protocol (%)				
Antagonist	1050 (44.8)	997 (45.2)	648 (27.6)	466 (19.9)
Agonist	328 (14.0)	825 (35.2)	1399 (59.7)	1563 (66.7)
Progestogen ovarian stimulation	288 (12.3)	323 (13.8)	267 (11.4)	314 (13.4)
Mild stimulation	678 (28.9)	199 (8.5)	30 (1.3)	1 (0.1)

Table 1. Demographics and cycle characteristics of patients[a]. The baseline characteristics, including age, infertility duration, BMI, basal FSH, basal LH, basal P, FSH on the day of trigger, LH on the day of trigger, P4 on the day of trigger, fertilization method, infertility diagnosis, and ovarian stimulation protocol, showed significant differences across the four groups (all $p < 0.001$). Abbreviation: BMI, body mass index, calculated by dividing weight in kilograms by the square of height in meters; IQR, interquartile range; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection. [a]Percentages may not sum to 100% due to rounding. [b]IVF is a complex series of procedures used to assist with the conception of a child; ICSI is a procedure where a single sperm is injected directly into an oocyte to achieve fertilization. [c]Infertility diagnosis (note that one patient may have multiple diagnoses): diminished ovarian reserve indicates a reduced capacity of the ovaries to produce oocytes; male factor refers to related to sperm concentration or function that impair the ability of sperm to fertilize an oocyte under normal conditions; tubal factor refers to obstruction or damage to the fallopian tubes; recurrent pregnancy failure defined as the occurrence of two or more consecutive miscarriages; ovulatory dysfunction refers to the inability of the varies to produce oocytes regularly; pelvic inflammatory disease refers to an infection affecting the female reproductive organs, typically involving the uterus, fallopian tubes, and ovaries, and in severe cases, can extend to the peritoneum; uterine factor, infertility due to structural or functional abnormalities of the uterus; other includes diagnoses that do not fit into the aforementioned categories, such as women with known chromosomal rearrangements or a history of malignancies leading to infertility. [d]Antagonist protocol, uses medication to quickly suppress the luteinizing hormone (LH) surge, preventing premature ovulation; Agonist protocol, uses medication to first stimulate and then suppress the pituitary gland, creating a controlled environment for follicle development; progestogen ovarian stimulation involves the use of progestogens, such as medroxyprogesterone acetate (MPA), in combination with gonadotropins to control the ovarian stimulation process; mild stimulation, uses lower doses of stimulating drugs over a shorter period to produce fewer, but potentially higher-quality eggs, reducing medication side effects and costs.

group (adjusted OR = 8.99, 95% CI: 5.75–14.08, $p < 0.001$) compared to the low response group, suggesting an independent association between E2 response levels and blastocyst formation rate (Supplementary Table 1).

Supplementary Tables 2 and 3 display the embryological outcomes of oocytes sourced from patients younger than 35 years or older, respectively. Supplementary Fig. 2 illustrates that the trend in blastocyst formation rate per follicle puncture is similar across these two age groups. Supplementary Tables 4 and 5 show the embryological outcomes of oocytes sourced from patients with BMI below 25 and those with a BMI of 25 or higher, respectively. Supplementary Fig. 3 indicates that trend in blastocyst formation rate follicle puncture is also similar across these two BMI groups. Regarding of age or BMI, the blastocyst formation rate steadily increased with higher E2 response.

Pregnancy outcomes

The four E2 response groups were comparable in terms of age, hormone levels, and infertility diagnosis, with no differences in infertility duration or fertilization method. A total of 4,456 fresh transfer cycles were confirmed, including (17.5%) in the low response group, 1,222 (27.4%) in the moderate response group, 1,415 (31.8%) in the moderate-high response group, and 1,041 (23.4%) in the high response group (Table 3). When simplified into two E2 response groups (low and high response), a chi-square test demonstrated a significant difference in clinical pregnancy rates between the two groups (0.401 vs. 0.575; $p < 0.001$).

As shown in Table 4, the clinical pregnancy rate was significantly higher in the moderate-high response group and high response group compared to the low response group (54.5% and 61.5% vs. 35.5%; absolute differences of 19% and 26%, respectively; adjusted relative risks [aRR] of 1.21 [95% CI: 1.03–1.42] and 1.27 [95% CI: 1.08–1.51], respectively). These differences remained significant after adjusting for age, baseline FSH, LH and P4 levels on the day of hCG, infertility diagnosis, ovarian stimulation protocols, and day of transfer. Given the significant differences in embryo transfer type (Day 3 vs. Day 5/6) among E2 response groups, we conducted an interaction analysis to evaluate its potential modifying effect on clinical pregnancy outcomes. The results showed no significant interactions (e.g., moderate-high response group: Day5, Estimate = −0.2261, $p = 0.278$; high response group: Day5, Estimate = −0.2209, $p = 0.294$), indicating that the associations between E2 response and clinical pregnancy outcomes are consistent across embryo transfer types. There was no significant difference in clinical pregnancy rate between the moderate response group and the low response group after controlling for confounders (aRR: 1.07 [95% CI: 0.91–1.25]). The live birth rate in the moderate-high response group and high response group was also significantly higher than in the low response group (44.9% and 52.0% vs. 25.7%; absolute differences of 19.2% and 26.3%; aRRs of 1.27 [95% CI: 1.06–1.52] and 1.35 [95% CI: 1.11–1.64],

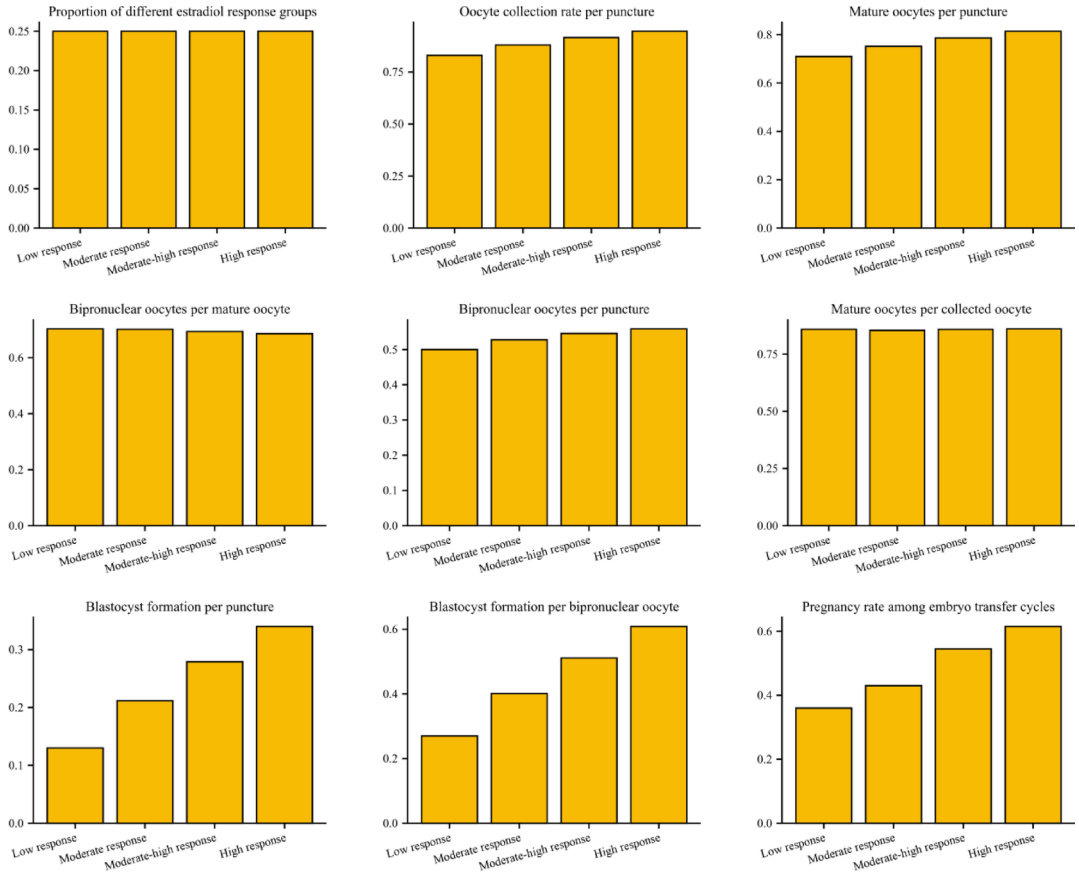

Fig. 2. Follicle punctures and embryology outcomes according to relative changes in serum E2 levels during COS.

E2 response rate	Follicle punctures (n)	Oocytes		Mature (M2) oocytes			2pn oocytes			Blastocyst formation		
		Total	PPF	Total	PPF	Per oocyte	Total	PPF	Per M2 oocyte	Total	PPF	Per 2pn oocyte
Low	8446	7036	0.83	6018	0.71	0.86	4232	0.50	0.70	1140	0.13	0.27
Moderate	16,195	14,253	0.88	12,175	0.75	0.85	8546	0.53	0.70	3428	0.21	0.40
Moderate-high	27,286	24,983	0.92	21,460	0.79	0.86	14,886	0.55	0.69	7609	0.28	0.51
High	41,436	39,191	0.95	33,734	0.81	0.86	23,147	0.56	0.69	14,089	0.34	0.61
All	93,363	85,463	0.92	73,387	0.79	0.86	50,811	0.54	0.69	26,266	0.28	0.52

Table 2. Oocyte recovery and embryology outcomes according to E2 response rate. Note: M2 = metaphase 2; 2pn = bipronuclear; PPF = per punctured follicle.

Characteristics median (IQR)	Low response (*n* = 778)	Moderate response (*n* = 1222)	Moderate-high response (*n* = 1415)	High response (*n* = 1041)
Age, y	38.0 (34.0–41.0)	36.0 (32.0–39.0)	34.0 (31.0–37.0)	33.0 (30.0–36.0)
Infertility duration	4.0 (2.0–8.0)	4.0 (2.0–7.0)	4.0 (2.0–7.0)	4.0 (2.0–6.0)
BMI	22.1 (20.4–24.1)	22.2 (20.2–24.6)	21.9 (20.2–24.3)	21.6 (19.7–23.8)
Basal FSH	6.4 (4.9–9.0)	5.9 (4.9–7.3)	5.5 (4.7–6.5)	5.2 (4.6–6.1)
Basal LH	3.0 (2.1–4.3)	2.9 (2.1–4.1)	3.0 (2.2–3.9)	3.1 (2.4–4.0)
Basal P4	0.2 (0.2–0.5)	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.2 (0.2–0.3)
FSH on trigger day	13.1 (10.1–16.4)	14.2 (11.3–17.0)	13.7 (10.8–17.0)	13.1 (10.3–16.3)
LH on trigger day	1.8 (1.0–3.4)	1.2 (0.7–2.1)	0.9 (0.7–1.5)	0.9 (0.7–1.3)
P4 on trigger day	0.2 (0.2–0.4)	0.3 (0.2–0.4)	0.4 (0.3–0.6)	0.5 (0.4–0.7)
Fertilization method				
IVF	584 (75.1)	900 (73.6)	1057 (74.7)	801 (76.9)
ICSI	194 (24.9)	322 (26.4)	358 (25.3)	240 (23.1)
Infertility diagnosis, No. (%)				
Diminished ovarian reserve	392 (50.4)	284 (23.2)	99 (7.0)	13 (1.2)
Male factor	74 (9.5)	185 (15.1)	248 (17.5)	160 (15.4)
Tubal factor	16 (2.1)	187 (15.3)	260 (18.4)	168 (16.1)
Recurrent pregnancy failure	12 (1.5)	20 (1.6)	31 (2.2)	15 (1.4)
Pelvic inflammatory disease	182 (23.4)	444 (36.3)	697 (49.3)	614 (59.0)
Uterine factor	33 (4.2)	76 (6.2)	74 (5.2)	57 (5.5)
Others	69 (8.9)	170 (13.9)	226 (16.0)	158 (15.2)
Ovarian stimulation protocol (%)				
Antagonist	409 (52.6)	559 (45.7)	373 (26.4)	206 (19.8)
Agonist	240 (30.8)	613 (50.2)	1029 (72.7)	835 (80.2)
Mild stimulation	129 (16.6)	50 (4.1)	13 (0.9)	0
Day of transfer No. (%)[a]				
Day 3	721 (92.7)	1000 (81.8)	940 (66.4)	518 (49.7)
Day 5	55 (7.1)	211 (17.3)	464 (32.8)	513 (49.3)
Day 6	2 (0.3)	11 (0.9)	11 (0.8)	10 (1.0)
No. of embryos transferred	2.0 (1.0–2.0)	2.0 (1.0–2.0)	2.0 (1.0–2.0)	1.0 (1.0–2.0)

Table 3. Demographic and cycle characteristics of transfer cycles. Age (years), BMI (kg/m²), Basal FSH (mIU/mL), Basal P4 (ng/mL), FSH on trigger day, LH on trigger day, P4 on trigger day, Infertility diagnosis, Ovarian stimulation protocol, and Day of transfer showed statistically significant differences among the groups, with $p < 0.001$. [a]Embryo transfer can occur at the cleavage stage (Day 3) or the blastocyst stage (Day 5/6). Blastocyst transfers typically result in higher pregnancy rates.

respectively). Although the live birth rate in the moderate response group was higher than in the low response group (34.5% vs. 25.7%; absolute difference of 8.8% [95% CI: −3.9–21.5%]; aRR of 1.11 [95% CI: 0.92–1.33]), the difference was not statistically significant. There were no significant differences in the miscarriage rate between the low response group and the moderate response group, moderate-high response group, and high response group (10.8%, 12.1%, 12.7% vs. 12.7%; absolute differences of 1.9%, 0.6%, and 0%; aRRs of 0.85 [95% CI: 0.65–1.33], 1.04 [95% CI: 0.78–1.39], and 1.21 [95% CI: 0.88–1.67], respectively). Similarly, there were no significant differences in ectopic pregnancy rate between the low response group and the other response groups.

Outcome	Event. No./total (%)	Absolute difference, % (95% Cl)	Relative risk (95% Cl)	
			Unadjusted	Adjusted[a]
Clinical pregnancy				
Low response	276/778 (35.5)	Reference	1 [Reference]	1 [Reference]
Moderate response	526/1222 (43.0)	7.5 (−6.0–21.0)	1.21 (1.08–1.36)	1.07 (0.91–1.25)
Moderate-high response	771/1415 (54.5)	19.0 (5.5–32.5)	1.54 (1.38–1.71)	1.21 (1.03–1.42)
High response	640/1041 (61.5)	26.0 (12.6–39.4)	1.73 (1.56–1.93)	1.27 (1.08–1.51)
Live birth				
Low response	200/778 (25.7)	Reference	1 [Reference]	1 [Reference]
Moderate response	421/1222 (34.5)	8.8 (−3.9–21.5)	1.31 (1.15–1.51)	1.11 (0.92–1.33)
Moderate-high response	636/1415 (44.9)	19.2 (6.2–32.2)	1.74 (1.54–1.99)	1.27 (1.06–1.52)
High response	541/1041 (52.0)	26.3 (13.3–39.3)	2.03 (1.79–2.31)	1.35 (1.11–1.64)
Miscarriage				
Low response	99/778 (12.7)	Reference	1 [Reference]	1 [Reference]
Moderate response	132/1222 (10.8)	1.9 (−10.8–7.0)	0.97 (0.76–1.23)	0.85 (0.65–1.33)
Moderate-high response	171/1415 (12.1)	0.6 (−0.97–8.5)	1.30 (1.04–1.62)	1.04 (0.78–1.39)
High response	132/1041 (12.7)	0 (−9.2–9.2)	1.56 (1.24–1.97)	1.21 (0.88–1.67)
Ectopic pregnancy				
Low response	5/778 (0.6)	Reference	1 [Reference]	1 [Reference]
Moderate response	11/1222 (0.9)	0.3 (−2.1–2.7)	1.58 (0.58–4.97)	1.49 (0.52–4.87)
Moderate-high response	15/1415 (1.1)	0.5 (−2.0–3.0)	2.36 (0.92–7.23)	2.36 (0.83–7.82)
High response	10/1041 (1.0)	0.4 (−2.1–2.9)	2.60 (0.93–8.30)	3.13 (0.95–11.53)

Table 4. Clinical pregnancy, live birth, miscarriage, and ectopic pregnancy rate in different E2 response rate. [a]Adjusted for age, baseline FSH, LH and P4 levels on the day of trigger, infertility diagnosis, ovarian stimulation protocols, and day of transfer.

Discussion

This study analyzed first ART cycles, classifying patients into four response groups based on the ratio of E2 levels on the day of hCG administration to baseline E2 during COS. E2 response levels correlated significantly with patient characteristics (age, BMI, baseline hormones) and key outcomes (oocyte retrieval, blastocyst formation rate, pregnancy rates). As E2 response levels increased, patient age decreased, ovarian function parameters (such as FSH and LH) improved, and oocyte retrieval and blastocyst formation rate significantly increased. Additionally, clinical pregnancy rate and live birth rate were significantly higher in the moderate-high response group and high response group compared to the low response group. These results suggest that E2 response level is an important predictive indicator for embryological outcomes and pregnancy success in ART cycles.

Estrogens regulate reproductive functions, with E2 being dominant during reproductive years. While estrogen's role in late pregnancy is well-studied, its function in early pregnancy is less understood[15,16]. In ART, exogenous hormones are used to modulate female sex hormone levels, mimicking the natural hormonal fluctuations of the menstrual cycle to optimize ART outcomes. However, the lack of high-quality studies has limited the establishment of standardized protocols[17]. Existing research on the role of E2 in ART has shown mixed results. Zavy et al.[18] divided patients into three groups based on E2 levels (< 2000 pg/ml, 2000–4000 pg/ml, > 4000 pg/ml) and found no significant differences in live birth rate or miscarriage rates between groups. Conversely, Joo et al.[19] stratified patients into five E2 level groups (< 1000 pg/ml, 1000–2000 pg/ml, 2000–3000 pg/ml, 3000–4000 pg/ml, > 4000 pg/ml) and observed significant differences in implantation rates, pregnancy rates, and live birth rate across these groups. The variation in E2 cutoff values used in different studies may explain the inconsistent findings. Differences in sample sizes may also contribute to these discrepancies. Larger studies, such as Kondapalli et al.[13] with 1712 patients and Wang et al.[20] with 3393 patients, have reported significant increases in live birth rate in higher E2 level groups, providing greater statistical power and more reliable results. In contrast, smaller studies by Zavy et al.[18] and Morales et al.[21], with 478 and 181 patients respectively, may have lacked the power to detect subtle associations between E2 levels and pregnancy outcomes.

In contrast to these studies, our research utilized the ratio of (trigger day E2 - baseline E2)/baseline E2) to categorize patients. This approach offers better control of individual baseline E2 differences and dynamically captures relative change in E2 levels, providing a more consistent evaluation metric. Previous studies have shown that E2 levels on the day of HCG administration can effectively predict oocyte retrieval, maturation, and subsequent fertilization[21]. Consistent with these findings, our study demonstrated a positive correlation between E2 response levels during COS and oocyte retrieval, maturation, and fertilization rates. After adjusting for confounding factors, the moderate-high response group and high response group still showed significantly better oocyte retrieval, maturation, and fertilization outcomes compared to the low response group. Some studies have suggested that E2 may negatively affect blastocyst formation, as seen in bovine oocyte in vitro maturation (IVM) studies where 1 μg/ml of E2 reduced nuclear maturation and blastocyst formation rate[22], others have reported that E2 promotes blastocyst formation in pigs[23]. Such differences may be attributed to variations in species, culture conditions, and E2 concentrations. Our data indicate a moderate positive correlation between

E2 response levels during COS and blastocyst formation rate, with significant differences observed between the low response group (0.13), moderate response group (0.212), moderate-high response group (0.279), and high response group (0.34). Even after adjusting for confounding factors, these differences continued to be significant. When the response groups were simplified into "low" and "high" response categories, the high response group had a significantly higher blastocyst formation rate than the low response group, underscoring the importance of E2 response in blastocyst development. Furthermore, both univariate and multivariate logistic regression analyses supported this conclusion, indicating a strong and independent association between E2 response and blastocyst formation. Blastocyst formation analysis (Supplemental Fig. 1) also showed similar trends between oocytes retrieved from patients aged ≥ 35 years and those aged < 35 years. Similarly, comparisons between BMI < 25 kg/m² and BMI ≥ 25 kg/m² (Supplemental Fig. 2) revealed similar trends, indicating that neither advanced age nor obesity significantly impacted the relationship between E2 response and blastocyst formation.

Estrogen plays a crucial role in endometrial receptivity by promoting epithelial proliferation, tissue regeneration, and enhancing implantation factors such as leukemia inhibitory factor (LIF) and MUC-1, which support embryo attachment and decidual invasion[24–26]. It also influences immune cells like uterine natural killer (uNK) cells and decidual macrophages, contributing to endometrial remodeling and creating a favorable implantation environment[27–29]. It also influences immune cells like uNK cells and decidual macrophages, contributing to endometrial remodeling and creating a favorable implantation environment[30,31]. Therefore, investigating E2 levels may provide valuable insights into understanding endometrial receptivity and its role in ART.

Notably, maintaining E2 levels within an optimal range during ART is crucial for achieving favorable reproductive outcomes. Studies suggest that both high and low E2 levels may negatively impact pregnancy outcomes, with higher E2 linked to reduced live birth rate and increased ectopic pregnancies[32,33]. High E2 levels have also been linked to adverse neonatal outcomes, such as low birth weight[5], placenta-related complications[3], and preeclampsia[4], suggesting that elevated E2 levels may negatively impact pregnancy progression. Conversely, low E2 levels can also affect pregnancy outcomes. Chen et al.[34] reported that E2 levels < 1200 pg/ml were associated with an increased risk of preeclampsia, and lower E2 levels might reduce endometrial receptivity, affecting embryo implantation and pregnancy maintenance[35].

In our study, based on the ratio of trigger day E2 to baseline E2 during COS, we found that as the E2 response ratio increased, clinical pregnancy rate and live birth rate significantly improved. The moderate-high response group and high response group had significantly higher clinical pregnancy rate and live birth rate compared to the low response group, and these differences remained significant after adjusting for potential confounders. However, for the moderate response group, although clinical pregnancy rate and live birth rate were higher than in the low response group, the differences were not statistically significant after adjusting for confounders, indicating that lower E2 response levels may not be sufficient to impact pregnancy outcomes. Furthermore, although miscarriage rate in the moderate-high response group and high response group showed statistical differences before adjustment, the absolute differences with the low response group were small, and after adjusting for confounders, these differences were not significant. Similarly, no significant differences in ectopic pregnancy rate were observed between the E2 response groups. Given the similar miscarriage rate and ectopic pregnancy rate across E2 response groups, differences in live birth rate may be attributed to differences in implantation rates rather than pregnancy loss. These data suggest that E2 response levels during COS may influence embryo implantation success. Although no differences in ectopic pregnancy rate were observed between the low and high response groups, the wide confidence intervals reflect the variability of such rare outcomes in this large cohort.

The findings of this study have important implications for clinical practice. To our knowledge, this is the largest study to explore the impact of E2 response during COS on embryo development and pregnancy outcomes. Based on a large dataset of 9376 fresh, first IVF/ICSI cycles, we provide a comprehensive analysis of E2 response as a predictor of embryo development and pregnancy outcomes in ART. Using the ratio of trigger day E2 to baseline E2 offers a dynamic and individualized assessment of E2 changes during COS, improving predictive accuracy over traditional absolute E2 measurements. This method, compared to traditional analyses based on absolute E2 values, allows for better control of individual variability and provides clinicians with a more reliable predictive tool for assessing patients' responses to COS and forecasting embryo development and pregnancy outcomes. Additionally, the multivariate analysis in our study controlled for several key confounding factors, such as age, baseline FSH, LH, and P4 levels on the day of hCG, infertility diagnosis, and stimulation protocols, enhancing the reliability of our findings. We observed a significant association between E2 response and blastocyst formation rate, as well as live birth rate, and these associations remained significant even after adjusting for important covariates. This suggests that the relative change in E2 response could serve as an independent predictor of clinical outcomes, offering important clinical utility.

Despite the strengths of our study, there are some limitations to consider. First, as a retrospective analysis, the study design cannot establish causality but demonstrates only an association between E2 response and pregnancy outcomes. Future prospective studies are needed to confirm our findings and explore the causal mechanisms behind them. Second, although we assessed the E2 response using a ratio to reflect the relative changes during COS, the thresholds for categorizing E2 response groups were based on the quartile method from the cohort's data distribution. While statistically robust, these thresholds lack established clinical cutoffs and require further validation in future studies. Additionally, the clinical applicability of this ratio still requires validation, especially across different populations and stimulation protocols, as variations in baseline E2 levels and individual physiological responses could impact its generalizability. Third, although we adjusted for ovarian stimulation protocols in our multivariable analysis, the inherent heterogeneity in these protocols may still influence outcomes. Different protocols create distinct hormonal environments that can affect both embryological outcomes and endometrial receptivity, especially in fresh embryo transfers, where endometrial

conditions are more sensitive to supraphysiological hormone levels. Additionally, as this study focused exclusively on fresh IVF/ICSI cycles, the applicability of our findings to frozen-thawed embryo transfer (FET) cycles remain uncertain, which may limit the generalizability of our results. Fourth, patients with PCOS and endometriosis were excluded to reduce heterogeneity, but this limits the applicability of our findings to these subgroups, warranting further investigation. Finally, although we adjusted for several confounding factors, there may still be residual confounders, such as patient lifestyle, metabolic factors, or subtle differences in stimulation protocols, that could influence pregnancy outcomes but were not fully captured in our dataset.

Conclusion

In this retrospective cohort study, the relative change in E2 response during COS was significantly associated with blastocyst formation rate, clinical pregnancy rate, and live birth rate. Higher E2 responses were predictive of improved pregnancy outcomes. However, the interpretation of these results is limited by the retrospective design and potential biases due to selection and residual confounding. Future prospective studies are needed to further validate the predictive value of E2 response and to identify the optimal range of E2 levels for maximizing pregnancy success.

Data availability

The data that support the findings of this study are not publicly available due to privacy concerns regarding patient confidentiality but may be made available from the corresponding author upon reasonable request.

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Conceptualization, Wenjie Huang, Jingjing Li and Li Fan; Formal analysis, Wenjie Huang, Liuyan Wei, Juan Tang, Liuying Nong and Ni Tang; Funding acquisition, Jingjing Li and Li Fan; Investigation, Qiuyue Wen and Lixiang Xu; Methodology, Liuyan Wei, Liuying Nong and Ni Tang; Supervision, Jingjing Li and Li Fan; Validation, Qiuyue Wen, Zuxing Qin and Lixiang Xu; Writing – original draft, Wenjie Huang; Writing – review & editing, Jingjing Li and Li Fan.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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