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RESEARCH

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Effect of estradiol supplementation on luteal support following a significant reduction in serum estradiol levels after hCG triggering: a prospective randomized controlled trial

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Abstract

Objective This study aimed to evaluate the impact of adding 4 mg estradiol valerate to progesterone for luteal support on pregnancy rates in IVF cycles following a long protocol with reduced luteal serum estradiol levels post-hCG triggering.

Design, setting, and participants The prospective randomized controlled trial was conducted at a public tertiary hospital reproductive center with 241 patients who experienced a significant decrease in serum estrogen levels post-oocyte retrieval.

Interventions Participants received either a daily 4 mg dose of estradiol valerate in addition to standard progesterone or standard progesterone alone for luteal support.

Results The ongoing pregnancy rate did not show a significant difference between the E2 group and the control group (56.6% vs. 52.2%, with an absolute rate difference (RD) of 4.4%, 95% CI -0.087 to 0.179, $P=0.262$). Similarly, the live birth rate, implantation rate, clinical pregnancy rate, early abortion rate, and severe OHSS rate were comparable between the two groups. Notably, the E2 group had no biochemical miscarriages, contrasting significantly with the control group (0.0% vs. 10.7%, RD -10.7%, 95% CI -0.178 to -0.041, $P=0.000$). In the blastocyst stage category, the clinical pregnancy rate was notably higher in the E2 group compared to the control group (75.6% vs. 60.8%, RD 14.9%, 95% CI 0.012 to 0.294, $P=0.016$).

Conclusion Adding 4 mg estradiol valerate to progesterone for luteal support does not affect the ongoing pregnancy rate in embryo transfer cycles using a long protocol with a significant decrease in serum estradiol levels after hCG triggering. However, it may reduce biochemical miscarriages and positively impact clinical pregnancy rates in blastocyst embryo transfer cycles.

Trial registration ChiCTR1800020342.

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Keywords Luteal phase support, Estradiol supplementation, Serum estradiol drop, Ongoing pregnancy rate

Introduction

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are widely acknowledged treatments for addressing various types of infertility. Among the stimulation protocols used in these treatments, the agonist long protocol is commonly employed. However, some patients undergoing this protocol experience a notable decrease in serum estradiol (E2) levels following human chorionic gonadotropin (hCG) triggering. This reduction in E2 could potentially impact the treatment's success.

E2 plays a crucial role in stimulating the proliferation of endometrial cells in the basal layer and increasing progesterone receptors. Progesterone, in turn, prompts the secretion of endometrial gland cells and the decidualization of the stromal layer. Therefore, optimal concentrations of both estrogen and progesterone are necessary for the adequate maturation of the endometrium before embryo implantation [1] (Lessey and Young, 2014). As estrogen operates through paracrine/autocrine signaling rather than direct regulation, it must reach a certain threshold to initiate morphological and biological changes that favor endometrial receptivity for embryo attachment and implantation maintenance [2]. In assisted reproduction cycles, serum estradiol levels reach their peak during follicular maturation. However, supraphysiological estrogen levels experience a significant decline after oocyte retrieval via follicular aspiration. It has been established that this decline adversely affects the implantation rate [3, 4]. A study by Xueyan Bai et al. [5] revealed that an 80% reduction in E2 levels post-oocyte retrieval led to a decrease in the pregnancy rate from 51.33 to 36.72% and the implantation rate from 30.93 to 21.70% in high ovarian responders.

The practice of supplementing estradiol with progesterone to support the luteal phase in IVF/ICSI cycles has sparked controversy. While some studies suggest that E2 supplementation might enhance implantation rates [6], this assertion lacks validation in a meta-analysis study [7]. Importantly, the absence of definitive evidence from prospective randomized controlled trials regarding the use of E2 supplementation is notable, primarily due to the variability of serum estradiol levels, particularly following a rapid decline post-HCG triggering.

Therefore, a prospective randomized controlled trial was devised to determine whether adding estradiol to progesterone for luteal phase support could improve clinical pregnancy outcomes in cycles utilizing the agonist long protocol with a marked decrease in luteal serum E2 after HCG triggering.

Materials and methods

Participants

This randomized controlled trial (RCT) took place at the Assisted Reproduction Centre of Northwest Women's and Children's Hospital, China, from March 2019 to January 2020. Women undergoing IVF/ICSI cycles were invited to participate in the study. Inclusion criteria were as follows: age < 40 years, utilization of the agonist long protocol, fresh cycle embryo transfer, and a serum estradiol level decrease of > 60% from the hCG trigger day to 3 days after oocyte retrieval. Exclusion criteria included an endometrial thickness < 8 mm, uterine malformation, endometriosis, and a peak serum estradiol level ≥ 5000pg/ml. All patients provided informed consent, and the study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Northwest Women's and Children's Hospital. Additionally, the study was registered in the Chinese Clinical Trial Registry under Registration No. ChiCTR1800020342. Reporting of the study adhered to CONSORT guidelines.

The flow of participants is illustrated in Fig. 1. Initially, a total of 1160 patients were considered for recruitment, but 915 were excluded due to not meeting the inclusion criteria (estradiol decline rate < 60%). In the final analysis, 20.68% of the screened patients exhibited an estradiol decline rate ≥ 60%. Four patients declined to participate, and 26 patients who consented were not randomized due to reasons such as unavailability of embryos for embryo transfer, risk of ovarian hyperstimulation syndrome (OHSS), or other factors. Ultimately, 219 patients completed the full allocated intervention and were randomized into the study group (E2 group, $n = 106$) and the control group (Control group, $n = 113$).

Stimulation regimen

All participants followed the agonist-long protocols, followed by the in-vitro fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) regimen.

For those undergoing the long-term agonist protocol, subcutaneous administration of 0.1 mg triptorelin acetate (Decapeptyl, Ferring Ltd., Wittland, Germany) began in the mid-luteal phase of the preceding menstrual cycle and continued for 14 days.

After undergoing a baseline ultrasound scan, patients were deemed to have achieved a fully downregulated state when the following criteria were met: serum estradiol (E2) levels were below 50 pg/ml, luteinising hormone (LH) levels were less than 5 IU/ml, the endometrial thickness was less than 5 mm, and the diameter of the largest follicle fell within the range of 5–10 mm.

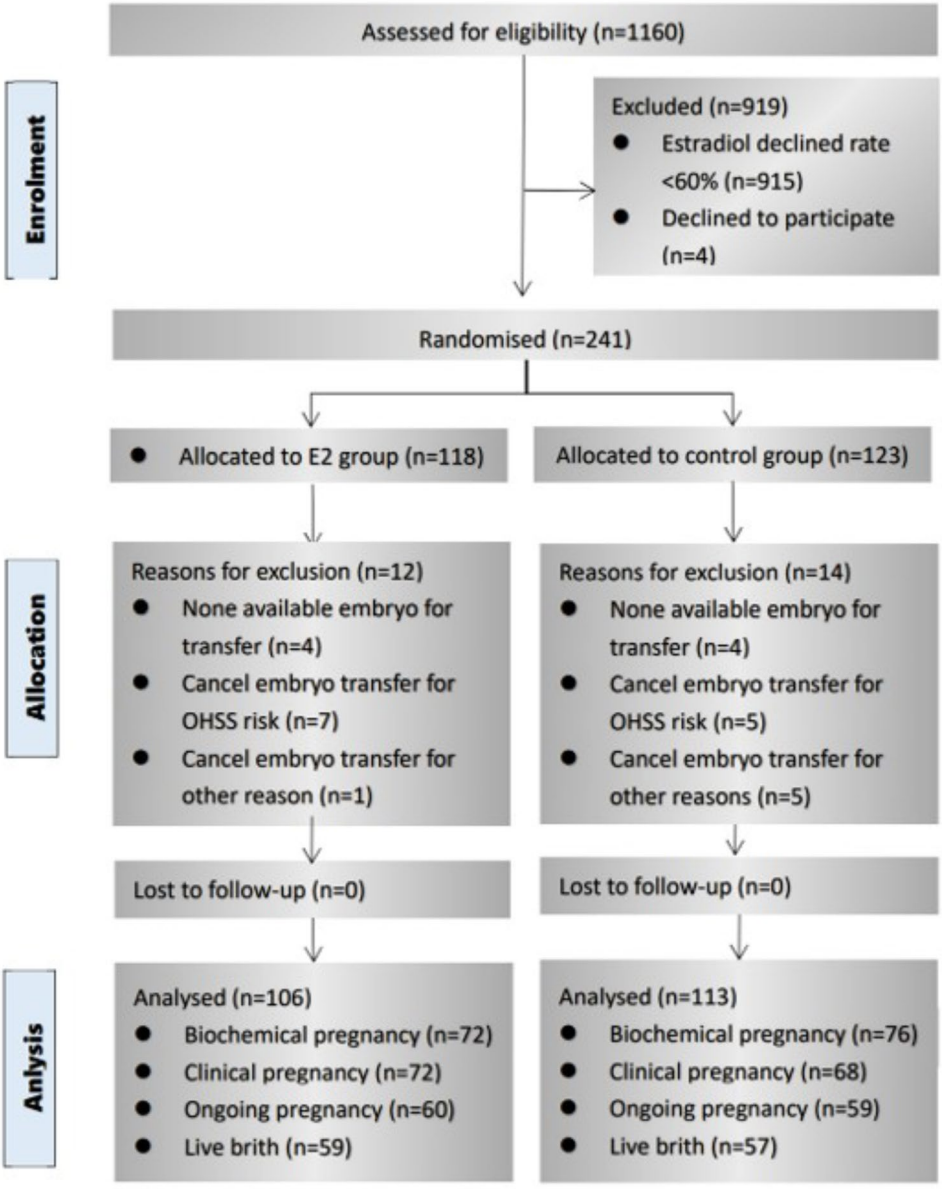

Fig. 1 Flowchart

After achieving downregulation, recombinant follicle-stimulating hormone (rFSH, Gonal-F, Merck) was administered at a dosage ranging from 150 to 225 IU per day. When more than two follicles reached a diameter exceeding 18 mm, an injection of 6,500 to 10,000 units of Human Chorionic Gonadotropin (hCG) (brands such as Ovidrel, Merck; or Livzon) was administered.

Oocytes were retrieved via transvaginal ultrasound-guided follicular aspiration 36 h post-hCG injection, and the number of retrieved oocytes was recorded.

Blood samples and hormone measurements

Blood tests were conducted in the morning from 7:30 to 9:30. We routinely measure serum E2 levels on the day of hCG trigger, and in this study, we re-measured serum E2 levels on the third day after oocyte retrieval (as shown in Fig. 2), calculating the decline in E2 levels after retrieval by the difference between the latter and the former. Serum E2 concentrations were measured using an electrochemiluminescence immunoassay kit (Beckman Coulter, USA), with a minimum detection limit of 15 pg/mL. The intra- and inter-assay coefficients of variation were 4.3% and 5.5% for the low control and 5.1% and 7% for the high control, respectively.

IVF/ICSI-ET

Oocytes were retrieved and fertilized using either the conventional method or intracytoplasmic sperm

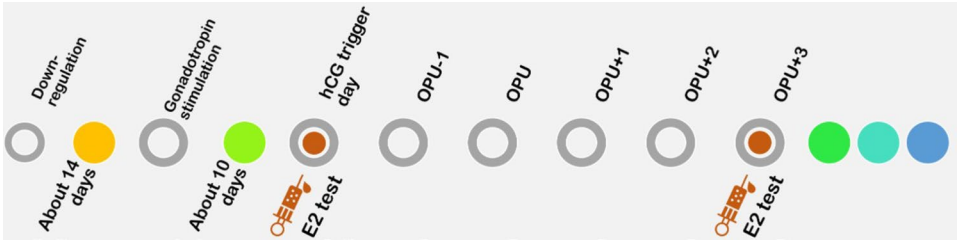

Fig. 2 Research technical route. Ovum Pick Up (OPU), Blood draw for serum estradiol testing: (E2 test)

injection, depending on sperm quality. One to two embryos with the highest quality were selected for transfer on day 3 or 5. If more than two good quality embryos were available, they were cultured to the blastocyst stage, and single blastocyst transfer was preferred.

Randomization Procedure

Random numbers were generated using a computer in a 1:1 ratio, and these numbers were then sealed in opaque envelopes. A postgraduate student, who was not involved in clinical work and was unaware of patient details, was tasked with grouping the patients randomly. Randomization occurred on the 3rd day after oocyte retrieval. Patients in both the trial and control groups were informed of their group assignments, which were also noted by clinicians. However, the embryologists and statisticians remained blinded to the group allocations.

Sample size calculation

According to the optimization scheme, to achieve a 15% increase in the clinical pregnancy rate with 80% power and α set at 0.05, a sample size of 150 was determined for each group. Participant dropouts at baseline were not accounted for, with the reliability of the center's follow-up system being the main consideration.

Luteal phase support

Serum estradiol levels were assessed in all enrolled patients on the hCG trigger day and 3 days after ovum pick up (OPU). If the E2 drop rate [(E2 level of OPU^{+3} day – E2 level of hCG trigger day)/ E2 level of hCG trigger day ×100%] exceeded 60%, patients were randomly assigned to two groups. The control group received conventional corpus luteum support, including progesterone injection at 60 mg/d intramuscularly, along with dydrogesterone at 20 mg/d orally or Crinone vaginal gel at 90 mg/d combined with dydrogesterone at 20 mg/d orally. In addition to conventional luteal support treatment, the study group (E2 group) received estradiol valerate at 4 mg/d orally from the 3rd day after OPU until the day of the serum beta-HCG test. Serum beta-HCG concentration was tested 14 days after ET. If pregnancy was confirmed, the progestogen was continued, and the estradiol dose was reduced by 1/3 every 3 days until discontinuation. Progesterone was continued in pregnant patients until approximately 10 weeks' gestation to support the luteal phase.

Outcome measures

The primary outcome measure was the ongoing pregnancy rate, while secondary outcome measures included the implantation rate, early abortion rate, clinical pregnancy rate, live birth rate, and the incidence of severe OHSS.

Outcome variables

Biochemical pregnancy: Serum beta-hCG level > 25mIU/ml.

Biochemical miscarriage: Positive pregnancy test without ultrasound evidence of a gestational sac.

Clinical pregnancy: Positive serum beta-hCG test result with ultrasound evidence of a gestational sac and fetal heart.

Implantation rate: Number of gestational sacs with fetal hearts assessed by ultrasound at 6–7 weeks' gestation divided by the number of embryos transferred.

Ongoing pregnancy: Pregnancy progressing beyond 12 weeks' gestation.

First-trimester pregnancy loss: Miscarriage after ultrasound evidence of an embryonic sac with or without a fetal pole not beyond 12 weeks.

Live birth: Birth of at least one newborn after 24 weeks' gestation exhibiting any sign of life (twins counted as a single birth).

Statistical analysis

Data were analyzed using the statistical software packages R (The R Foundation; http://www.r-project.org; version 4.2.0) and EmpowerStats (www.empowerstats.net, X&Y Solutions, Inc. Boston, Massachusetts). Quantitative variables were presented as mean (standard deviation, SD), with the number of observations (N) provided. Categorical variables were expressed as number (percentage). The Kruskal-Wallis Rank Test was utilized for continuous variables, while Fisher's Exact Test was applied for categorical variables with expected counts less than 10. The rate or mean difference between the groups, along with the 95% confidence interval (CI) and

the corresponding P-value, were calculated using a Generalized Linear Model. A P-value < 0.05 was considered statistically significant.

Results

The baseline characteristics of the patients are presented in Table 1. Both the E2 administration group (n=106) and the control group (n=113) exhibited comparable baseline characteristics. In the E2 group, the mean ages of females and males were 31.0 (3.5) and 32.8 (4.7) years, respectively, while in the control group, they were 31.1 (3.7) and 32.8 (4.8) years, respectively. Mean female BMI, smoking prevalence, duration of infertility, proportion of primary infertility, and causes of infertility were also similar between the two groups.

The distribution of education levels among females and the mean antral follicle count did not significantly differ between the groups. Additionally, the mean basal FSH level was 6.7 IU/l in both groups. All comparisons resulted in non-significant p-values, indicating no statistically significant differences in these baseline characteristics between the two groups, thereby ensuring their comparability for further analysis.

No significant differences were observed between the E2 administration and control groups regarding total gonadotrophin dose (P=0.271), total Gn days (P=0.599), hormone levels on hCG day (E2 P=0.764, P P=0.347, LH P=0.296), endometrial thickness (P=0.151), number of oocytes retrieved (P=0.147), and E2 level on the 3rd day post-OPU (P=0.678).

Fertilization method (P=0.390), number of embryos transferred (P=0.680), day of embryo transfer (P=0.546), and inclusion of at least one top-quality embryo (P=0.692) were also comparable between groups. These findings suggest similar effectiveness of E2 administration and control treatments in IVF outcomes. Further details are provided in Table 2.

The pregnancy outcomes were compared between the E2 administration and control groups, as shown in Table 3. No significant differences were observed in the biochemical pregnancy rate (67.9% vs. 67.3%, P=0.464), clinical pregnancy rate (67.9% vs. 60.2%, P=0.111), implantation rates (60.8% vs. 55.9%, P=0.418), first trimester pregnancy loss (13.9% vs. 11.8%, P=0.491), ectopic pregnancy (2.8% vs. 1.5%, P=0.316), ongoing pregnancy rate (56.6% vs. 52.2%, P=0.262), live birth rate (55.7% vs. 50.4%, P=0.147), and premature delivery (11.9% vs. 10.5%, P=0.351).

However, the E2 group exhibited significantly lower rates of biochemical miscarriage (0% vs. 10.7%, P<0.001) and twins (3.4% vs. 15.8%, P=0.016), indicating a potential protective effect of E2 administration against these outcomes.

No significant differences were found in gestational days (271.5 vs. 268.9, P=0.257) and severe OHSS (0% vs. 0.9%, P=1.000). Overall, the results suggest comparable pregnancy outcomes between the two groups, except for biochemical miscarriage and twin rates.

In the subgroup analysis of pregnancy outcomes following D5 blastocyst transfer, the E2 administration group (n=78) demonstrated a higher clinical pregnancy

Table 1 Baseline characteristics of population

	E2 administration	control	*P*-value
	(n=106)	(n=113)	
Female Age (years), mean(SD)	31.0 (3.5)	31.1 (3.7)	0.869
Male age (years), mean(SD)	32.8 (4.7)	32.8 (4.8) 3	0.992
Female BMI (kg/m2), mean (SD)	22.6 (3.4)	22.1 (3.0)	0.241
Smoking, n (%)	4 (3.8%)	5 (4.4%)	0.808
Infertility years (years), mean(SD)	3.2 (2.3)	3.6 (2.5)	0.169
Primary infertility, n (%)	56 (52.8%)	64 (56.6%)	0.572
Causes of infertility			0.826
Tube factor, n (%)	46 (43.4%)	49 (43.4%)	
Male factor, n (%)	25 (23.6%)	33 (29.2%)	
Multiple factors, n (%)	17 (16.0%)	15 (13.3%)	
Others, n (%)	12 (11.3%)	12 (10.6%)	
Unexplained, n (%)	6 (5.7%)	4 (3.5%)	
Education dgree of female			0.279
Basic education, n (%)	37 (34.9%)	41 (36.3%)	
College education, n (%)	37 (34.9%)	45 (39.8%)	
Postgraduate, n (%)	32 (30.2%)	27 (23.9%)	
Antral follicle count, mean (SD)	12.7 (4.8)	12.5 (4.3)	0.721
base FSH (IU/l), mean (SD)	6.7 (1.6)	6.7 (1.5)	0.959

Kruskal Wallis Rank Test for continuous variables, Fisher Exact for categorical variables with Expects<10

Table 2 Outcmes of stimulation and IVF

	E2 administration	**control**	***P*-value**
	(*n*=106)	**(*n*=113)**	
Total dose of gonadotrophin (IU), mean(SD)	2407.7 (865.7)	2287.4 (746.2)	0.271
Total Gn days continuous(Day), mean(SD)	11.4 (2.6)	11.2 (2.4)	0.599
Hormone level and ultrasond on HCG day			
E2 (pg/ml), mean(SD)	3409.4 (1150.2)	3454.5 (1069.7)	0.764
P (ng/ml), mean(SD)	1.3 (0.6)	1.4 (0.5)	0.347
LH (IU/ml), mean(SD)	1.6 (1.0)	1.8 (1.1)	0.296
Endometrial thickness (mm), mean(SD)	12.5 (1.9)	12.1 (2.1)	0.151
No. of oocytes retrieved, **mean(SD)**	9.3 (3.7)	10.1 (3.9)	0.147
E2 level on 3rd day after OPU, **mean(SD)**	1065.5 (411.7)	1088.2 (397.4)	0.678
Fertilization method			0.390
In vitro fertilization, n (%)	84 (79.3%)	84 (74.3%)	
Micro insemination, n (%)	22 (20.8%)	29 (25.7%)	
No. of embryos transferred			0.680
One embryo, n (%)	82 (77.4%)	90 (79.7%)	
Two embryos, n (%)	24 (22.6%)	23 (20.4%)	
D3/D5 of embryos for transfer			0.546
D3 embryo, n (%)	28 (26.4%)	34 (30.1%)	
D5 embryo, n (%)	78 (73.6%)	79 (69.9%)	
Include at lest 1 top quality embyro, n (%)	98 (92.5%)	106 (93.8%)	0.692

Kruskal Wallis Rank Test for continuous variables, Fisher Exact for categorical variables with Expects<10

Table 3 Outcmes of pregnancy

	E2 administration	**control**	**Absolute rate difference (95% CI)**	***P*-value**
	(*n*=106)	(*n*=113)		
Biochemical pregnancy rate per transfer cycles, n (%)	72 (67.9%)	76 (67.3%)	0.67% (-0.121, 0.134)	0.464
Biochemical miscarriage, **n (%)**	0 (0.0%)	8 (10.7%)	-10.7% (-0.178, -0.041)	0.000
Clinical pregnancy rate per transfer cycles, n (%)	72 (67.9%)	68 (60.2%)	7.8% (0.047, 0.209)	0.111
Implant rates, n (%)	79/130 (60.8%)	76/136 (55.9%)	4.9% (-0.174, 0.082)	0.418
Frist trimester pregnancy loss, n (%)	10 (13.9%)	8 (11.8%)	2.1% (-0.089, 0.134)	0.491
Eptopic pregnancy, n (%)	2 (2.8%)	1 (1.5%)	1.3% (-0.031, 0.059)	0.316
Ongoing pregnancy rate per transfer cycles, n (%)	60 (56.6%)	59 (52.2%)	4.4% (-0.087, 0.179)	0.262
Second trimester Pregnancy loss, n (%)	1 (1.7%)	2 (3.4%)	-1.7% (-0.076, 0.036)	
Live brith rate per transfer cycles, n (%)	59 (55.7%)	57 (50.4%)	5.2% (-0.076, 0.193)	0.147
Singleton/ Twins			-12.4 (-0.242, -0.020)	0.016
Singleton, n (%)	57 (96.6%)	48 (84.2%)		
Twins, n (%)	2 (3.4%)	9 (15.8%)		
Premature delivery, n (%)	7 (11.9%)	6 (10.5%)	1.3% (-0.102, 0.129)	0.351
Gestational days, (SD)	271.5 (12.9)	268.9 (13.7)	2.6 (-3.415, 6.527)	0.257
Severe OHSS, n(%)	0 (0.0%)	1 (0.9%)	-1% (0.000, Inf)	1.000

The rate/ mean difference with 95% CI and the P-value between the two groups were obtained by Generalized linear mode

rate per transfer cycle compared to the control group (*n*=79) (75.6% vs. 60.8%, *P*=0.016).

Although not statistically significant, there was also a trend towards higher rates in the E2 group for biochemical pregnancy (75.6% vs. 69.6%, *P*=0.202), ongoing pregnancy (61.5% vs. 51.9%, *P*=0.089), and live birth (60.3% vs. 49.4%, *P*=0.076).

These findings suggest that E2 administration may improve clinical pregnancy rates in D5 blastocyst transfer (Table 4).

Discussion

Our study aimed to assess the effect of estradiol (E2) supplementation in agonist long protocol cycles following a notable decrease in serum E2 levels post hCG triggering. This prospective, randomized controlled trial took place at a public tertiary hospital reproductive center. We hypothesized that adding 4 mg of estradiol valerate to progesterone for luteal support might increase the chances of pregnancy in cycles utilizing an agonist long protocol, especially when there is a sharp decline in luteal serum E2 after hCG triggering.

Table 4 Subgruop analysis of pregnancy outcomes

D5 Blastocyst transfer	E2 administration	control	Absolute rate difference (95% CI)	*P*-value
	(*n*=78)	(*n*=79)		
Biochemical pregnancy rate per transfer cycles, n (%)	59 (75.6%)	55 (69.6%)	6.0% (-0.073, 0.194)	0.202
Clinical pregnancy rate per transfer cycles, n (%)	59 (75.6%)	48 (60.8%)	14.9% (0.012, 0.294)	0.016
Ongoing pregnancy rate per transfer cycles, n (%)	48 (61.5%)	41 (51.9%)	9.6% (-0.051, 0.252)	0.089
Live brith rate per transfer cycles, n (%)	47 (60.3%)	39 (49.4%)	10.9% (-0.042, 0.262)	0.076

The rate difference with 95% CI and the P-value between the two groups were obtained by Generalized linear model

A comprehensive examination of pregnancy outcomes between the E2 and control groups offers insights into the potential impact of E2 supplementation in long agonist protocol cycles. Most of the measured pregnancy outcomes, including biochemical pregnancy rate, clinical pregnancy rate, implantation rates, first trimester pregnancy loss, ectopic pregnancy, ongoing pregnancy rate, live birth rate, and premature delivery, did not show significant differences between the two groups. This suggests that the overall effect of E2 supplementation on these outcomes may be minimal in the context of a significant reduction in serum E2 post hCG triggering.

However, two specific outcomes, biochemical miscarriage and twin rates, were significantly lower in the E2 group. The notable decrease in biochemical miscarriage rates in the E2 group suggests a potential protective effect of E2 administration in the early stages of pregnancy. The reduced twin rate in the E2 group is another important finding, indicating that E2 supplementation might contribute to better regulation of embryo development and implantation, thereby decreasing the likelihood of twin pregnancies.

The subgroup analysis of pregnancy outcomes following D5 blastocyst transfer provided further insights into the potential advantages of E2 administration. Notably, the E2 administration group exhibited a higher clinical pregnancy rate per transfer cycle compared to the control group. This result suggests that E2 supplementation might be particularly beneficial in cases involving D5 blastocyst transfers.

Regarding safety, our findings suggest that for participants with peak estradiol levels not exceeding 5000pg/ml, the daily supplementation of 4 mg of estradiol valerate does not appear to increase the risk of developing severe OHSS (0.0% versus 0.9%, $P=1.000$). This indicates that including estradiol as part of luteal support is safe in populations without a high ovarian response.

According to meta-analyses, the addition of E2 to progesterone for luteal phase support in IVF/ICSI cycles does not enhance pregnancy rates, irrespective of whether the GnRH agonist or GnRH antagonist protocol is utilized [7, 8]. Additionally, a decrease in serum estradiol levels in a controlled superovulation regimen is predictive of poor pregnancy outcomes [4, 5]. Few randomized controlled trials (RCTs) have assessed the impact of adding estradiol in cycles utilizing the long agonist protocol with a sharp decline in serum luteal E2 after HCG triggering. Our study found no disparity in the ongoing pregnancy rate between the two groups. However, there were no instances of biochemical miscarriage in the E2 group (0.0% (0/72) in the E2 group, and 10.7% (8/76) in the control group, rate difference −10.7% (95% CI -0.178, -0.041; $P=0.000$)). In the blastocyst stage category, a significant disparity was observed between the two groups in the clinical pregnancy rate. The control group had a rate of 60.8% (48/79), while the treatment group had a rate of 75.6% (59/78) (risk difference 14.9%, 95% CI 0.012, 0.294, $P=0.016$). The study suggests that adding 4 mg estradiol in patients with a sharp decrease in luteal phase estradiol may reduce biochemical pregnancy loss and improve pregnancy outcomes in blastocyst stage embryo transfer cycles. A retrospective cohort study [9] indicated that in cycles with estradiol levels below 5000 pmol/L on the day of hCG triggering, E2 supplementation led to a significantly higher live birth rate (23.44% vs. 32.92%, OR=1.60 [95% CI 1.05 to 2.46]). Our study found no statistically significant difference in the ongoing pregnancy rate and live birth rate between the E2 group and the control group for all study populations. However, there was an absolute rate difference of 4.4% (56.6% vs. 52.2%) in the ongoing pregnancy rate and a 5.2% (55.7% vs. 50.4%) improvement in the live birth rate. It is anticipated that more significant differences between the two groups will be observed in the future as the sample size is expanded.

Estrogen, like progesterone, is a key hormone that regulates the development of the uterine lining. During the period when implantation of the embryo occurs, estrogens play a crucial role in preparing the uterus to accept the embryo by activating signaling pathways within the uterine lining [2]. Estrogens need to reach a certain level to start the physical and biological changes that make the uterine lining receptive to embryo implantation [10].

In agonist regimens, the pituitary hormone LH is suppressed. The significant loss of granulosa cells during the retrieval of oocytes leads to insufficient luteal support. Multiple studies have confirmed the necessity of progesterone for supporting the luteal phase in fresh embryo transfer cycles following oocyte retrieval [11–13]. After receiving superovulatory treatment, women typically

exhibit peak serum estradiol levels that are several times higher than those seen during natural ovulation. This coincides with the development of multiple corpora lutea in the body, which can maintain adequate estradiol levels during the process of embryo implantation. As a result, there is currently no evidence supporting the use of estrogen supplementation during this phase.

In a retrospective cohort study, IVF fresh cycles were classified based on E2 levels on Day 28 of the menstrual cycle. The research found a link between E2 levels ≤ 50 pg/mL and an increased risk of biochemical pregnancy loss [14]. Our study indicates that in cases of a sudden drop in estradiol levels after retrieval, administering external estradiol might help reduce the risk of biochemical pregnancy loss. This could be due to the rapid decline of endogenous estrogen levels below physiological thresholds or the impact of declining estrogen levels on estrogen receptors and subsequent signal transduction, affecting endometrial receptivity. Supplementation of exogenous estradiol could potentially address the issue of embryo implantation failure resulting from inadequate or relative estrogen deficiency. Continuous monitoring of serum estrogen levels post-embryo transfer or endometrial evaluation in cases of failure may offer valuable insights into the effects of estrogen deficiency.

The transcription of hCG RNA occurs at the eight-cell stage, and the embryo begins producing the protein before implantation [15]. hCG plays a crucial role in stimulating luteal cells to sustain estrogen and progesterone production, crucial for pregnancy maintenance [16]. Three days post-oocyte retrieval, the cleavage-stage embryo is implanted into the uterus. Embryonic hCG likely contributes to maintaining stable estrogen levels during this period. However, on the fifth day post-retrieval, when blastocyst-stage embryos are implanting, there's a continued decline in estrogen levels among those with reduced E2. This decline, particularly if involving low estradiol levels or sharp fluctuations, might disrupt successful embryo implantation. Supplementation with external estrogen could benefit this subgroup of patients at this stage.

Additionally, the lower incidence of twin pregnancies in the E2 group holds significant implications for managing multiple pregnancies in IVF/ICSI treatments. Multiple pregnancies entail heightened risks for both mothers and infants. By decreasing the likelihood of twins, E2 administration could improve the safety and success rates of IVF/ICSI procedures. The precise mechanism behind this observation remains somewhat unclear, potentially attributed to the small sample size of our study, particularly following the analysis of subgroups categorized by the number of transplanted embryos (see Supplementary table).

While this study offers valuable insights, it is important to acknowledge its limitations. Firstly, the study was conducted at a single center, potentially limiting the generalizability of the findings to broader populations. Secondly, the study focused on patients with a specific criteria of serum estradiol decrease exceeding 60%, representing a minority (21.1%) of the population. This selective inclusion may restrict the applicability of the results to all IVF/ICSI patients undergoing long agonist protocol cycles. Future research could explore the effects of E2 administration across different protocols and patient cohorts. Thirdly, the study suffered from a small sample size, exacerbated by unaccounted dropout cases, leading to a reduced sample size for analysis. Finally, the study did not investigate potential side effects or risks associated with E2 supplementation. Subsequent studies should assess the safety profile of E2 supplementation in this context.

In this prospective, randomized controlled study, we investigated the impact of estradiol supplementation in long agonist protocol cycles, specifically focusing on cases with a notable decrease in serum E2 levels post hCG triggering. Our results indicate that overall pregnancy outcomes were similar between the group receiving E2 supplementation and the control group, except for notable differences in biochemical miscarriage rates and clinical pregnancy rates in the blastocyst stage category.

The E2 administration group demonstrated significantly lower rates of biochemical miscarriage and higher clinical pregnancy rates in the blastocyst stage category, suggesting a potential protective effect of E2 supplementation against these outcomes. These findings have implications for clinical practice in IVF/ICSI treatments, potentially improving treatment success by reducing early pregnancy loss and enhancing clinical pregnancy rates following blastocyst stage embryo transfer.

Conclusions

In summary, our study provides initial evidence supporting the benefits of E2 administration in long agonist protocol cycles, particularly following a significant decline in serum E2 levels post hCG triggering. These findings may inform clinical decision-making and protocol optimization in IVF/ICSI treatments, while also indicating avenues for future research.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-024-01275-x.

Supplementary Material 1

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Author contributions
Conception: NL, YH, and HW, Design: ZS and YH, Acquisition of data: NL, YH, and HC. Analysis of data: NL and JZS. Interpretation of data: NL. and YH, Drafted the manuscript: NL and YH. Critically revised the manuscript: HW, JZS, LJF and HC. "The author(s) read and approved the final manuscript."

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
Ethical approval was obtained from the Ethics Committee of Northwest Women's and Children's Hospital, XiAn, China. Code: 2018027. All participants completed an informed consent form.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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