Association between serum estradiol level on the human chorionic gonadotrophin administration day and clinical outcome

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Abstract

Background: Estradiol, as an important hormone in follicular development and endometrial receptivity, is closely related to clinical outcomes of fresh *in vitro* fertilization embryo transfer (IVF-ET) cycles. The aim of this retrospective study was to evaluate the association between elevated serum estradiol (E_2) levels on the day of human chorionic gonadotrophin (hCG) administration and IVF-ET pregnancy and birth outcomes.

Methods: A total of 1771 infertile patients with their first fresh IVF-ET cycles were analyzed retrospectively between January 2011 and January 2016 in Peking University First Hospital. Patients were categorized by serum E_2 levels on the day of hCG administration into six groups: group 1 (serum E_2 levels $\leq$ 1000 pg/mL, $n = 205$), group 2 (serum E_2 levels 1001–2000 pg/mL, $n = 457$), group 3 (serum E_2 levels 2001–3000 pg/mL, $n = 425$), group 4 (serum E_2 levels 3001–4000 pg/mL, $n = 310$), group 5 (serum E_2 levels 4001–5000 pg/mL, $n = 237$), and group 6 (serum E_2 levels $>$ 5000 pg/mL, $n = 137$). The retrieved oocyte and MII oocyte numbers and implantation and clinical pregnancy rates of the groups were compared as the first objective of the study. For the 360 women with singleton births among all patients, the area under the corresponding receiver operating characteristic curve (ROC curve) was calculated to assess the predictive value of the E_2 change for the probability of low birth weight (LBW) infants as the second objective.

Results: The retrieved oocyte and MII oocyte numbers and implantation and clinical pregnancy rates gradually increased from groups 1 to 5 but decreased in group 6. The parameters of group 1 were statistically worse than those of the other groups, from group 2 to group 6 (the number of retrieved oocytes, $t = 13.096$, $t = 23.307$, $t = 23.086$, $t = 26.376$, $t = 19.636$, $P < 0.003$; the number of retrieved MII oocytes, $t = 10.856$, $t = 20.868$, $t = 21.874$, $t = 23.374$, $t = 19.092$, $P < 0.003$; the implantation rate, $\chi^2 = 12.179$, $\chi^2 = 22.239$, $\chi^2 = 23.993$, $\chi^2 = 23.344$, $\chi^2 = 16.758$, $P < 0.003$; the clinical pregnancy rate, $\chi^2 = 16.415$, $\chi^2 = 28.074$, $\chi^2 = 35.387$, $\chi^2 = 37.025$, $\chi^2 = 24.590$, $P < 0.003$). ROC analysis revealed that when a serum peak E_2 of 3148 pg/mL was used to predict LBW.

Conclusions: The results indicate that serum E_2 levels have a concentration-dependent effect on clinical outcomes. The optimal range of the E_2 level during a fresh IVF-ET cycle is 1000 to 3148 pg/mL.

Keywords: Estradiol; *in vitro* fertilization; Clinical pregnancy rate; Low birth weight

Introduction

In vitro fertilization-embryo transfer (IVF-ET), now the main component of assisted reproductive technology (ART), is the most effective method of all ART methods in helping infertile patients. In IVF, retrieving a higher number of oocytes is positively correlated with a high live birth rate, and thus, controlled ovarian hyper-stimulation (COH) is widely used. In COH cycles, serum estradiol (E_2) levels can be increased by more than ten-fold over the levels found during spontaneous cycles.[1] Previous studies have shown that E_2 plays a key role in the regulation of uterine preparation for embryo implantation, via the stimulation of endometrial proliferation[2] and enhancement of uterine and endometrial perfusion.[3] Adequate endometrial preparation is essential to achieve and maintain pregnancy. In the natural cycle, elevations in serum E_2 concentrations shortly after the time of ovulation reduce endometrial receptivity. However, the effect of exposure to such high E_2 conditions on the day of human chorionic gonadotrophin (hCG) administration in IVF treatment is still not clear. Recent evidence suggests that serum E_2 levels have a concentration-dependent effect on pregnancy and delivery rates.[4,5] By contrast, the studies by Zavy *et al* and Wang *et al* report that serum E_2 on the hCG administration day does not alter the pregnancy rate.[6,7] Based on these data, the importance of high E_2 levels on the day of hCG

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administration remains controversial in terms of IVF outcome. Therefore, the first objective of our study was to evaluate the effect of the serum E_2 level on the day of hCG administration on the outcome of IVF-ET after COH. Theoretically, high E_2 concentrations at the time of implantation can impair the endometrial response to trophoblast invasion, leading to abnormal placentation. In previous research, some investigators have reported that exposure to such high E_2 concentrations at the time of implantation during infertility treatment may have a negative effect on endometrial receptivity.[8,9] Additionally, recent studies have found that the supraphysiologic serum E_2 unique to COH during ART increases the risk of abnormal placentation and might be responsible for adverse outcomes, such as miscarriages, preeclampsia (PreE), and the delivery of small fetuses.[4,10,11] The second objective of this study was to evaluate the effects of serum E_2 levels on the day of hCG administration on perinatal outcomes to determine an optimal range for the E_2 level to achieve a successful pregnancy.

Methods

Ethical approval

This study was approved by the Institutional Ethics Committee of the Peking University First Hospital, and written informed consent was obtained from each subject.

Patient selection

This retrospective cohort study was conducted after obtaining institutional approval and only analyzed the data of patients with non-donor oocyte retrieval resulting in fresh embryo transfer (ET). A total of 2998 patients undergoing their first IVF cycles from January 2011 to January 2016 were reviewed. Of these, 453 patients were excluded because of incomplete cycles (no oocyte retrieval, no ET due to fertility preservation, a freeze-all approach due to ovarian hyper-stimulation syndrome, or a lack of fertilization), 449 patients were excluded because no top-quality embryos were produced (embryos were graded by their morphologic appearance under a light microscope according to the system described by Staessen *et al*[12]), 312 patients were excluded due to a fibroid uterus, adenomyosis or abnormal pregnancy history, and 13 patients were excluded because of congenital uterine anomalies. Ultimately, 1771 patients constituted our final study cohort. According to serum E_2 levels on the day of hCG administration, the patients were categorized into six groups: group 1 (serum E_2 levels $\leq$ 1000 pg/mL, $n = 205$), group 2 (serum E_2 levels 1001–2000 pg/mL, $n = 457$), group 3 (serum E_2 levels 2001–3000 pg/mL, $n = 425$), group 4 (serum E_2 levels 3001–4000 pg/mL, $n = 310$), group 5 (serum E_2 levels 4001–5000 pg/mL, $n = 237$), and group 6 (serum E_2 levels $>$ 5000 pg/mL, $n = 137$).

A total of 530 patients underwent fresh IVF-ET cycles resulting in live births during the study period. Among them, 108 (20.4%) patients were excluded because of multiple gestations, and 62 (11.7%) patients were excluded because of vanishing twins. In total, 360 live singleton births (pregnancy starting with single gestational sac and fetal heart beat on initial ultrasound at 6 weeks) remained. The flow chart in Figure 1 summarizes the selection of the study cohort.

Controlled hyper-stimulation induction and embryo transfer

The gonadotrophin-releasing hormone (GnRH) agonist long protocol and the GnRH antagonist protocol were used in the cycles for this study. The GnRH agonist (GnRH-a) long protocol consisted of daily injections of short-acting GnRH-a and of long-acting GnRH-a at different doses during the early follicular or mid-luteal phases.

In the case of the daily short-acting GnRH-a injections, patients received a daily injection of 0.1 mg Decapeptyl (Ferring AG, Dübendorf, Switzerland) from the mid-luteal

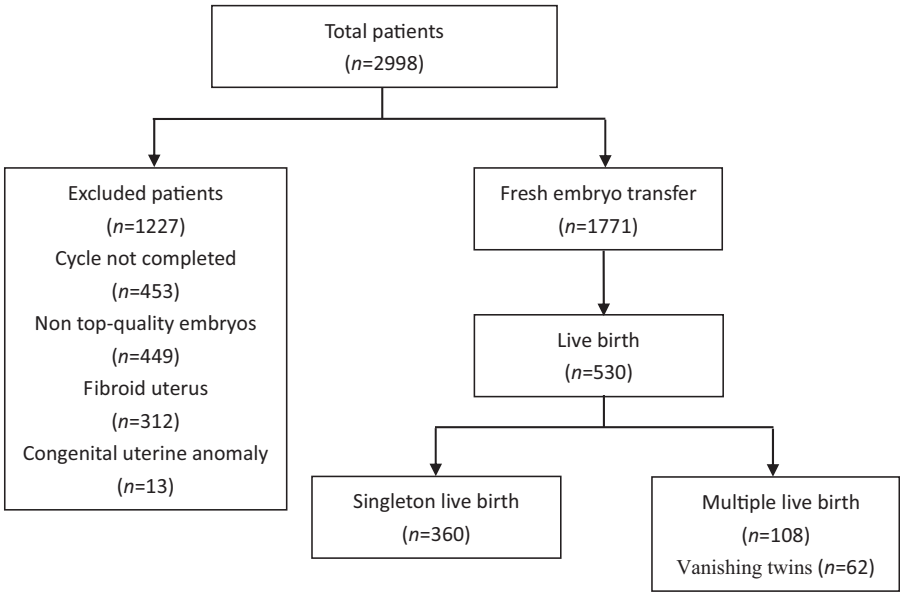

Figure 1: Overview of the inclusion and exclusion criteria.

phase of the pre-stimulation cycle, and the injections continued for approximately 15 to 18 days. Pituitary-ovarian suppression was confirmed with serum luteinizing hormone (LH) < 5 mIU/mL, E_2 < 50 pg/mL, antral follicle diameter ≤5 mm and endometrial thickness <5 mm. After ovarian suppression, the dose of Decapeptyl was reduced to 0.05 mg daily, and gonadotrophin was administered until the day of hCG administration.

During the administration of long-acting GnRH-a protocols for pituitary down-regulation, a single dose (3.75 mg) or a 1/4 dose (0.94 mg) of triptorelin (Ipsen Pharma Biotech, Signes, France) was injected during the early follicular period. After 21 to 28 days, following the confirmation of pituitary-ovarian suppression, gonadotrophin was injected. During treatment, the ovarian response was monitored with vaginal ultrasound measurements of follicular growth and the serum E_2 concentration.

The GnRH antagonist protocol consisted of daily gonadotrophin stimulation from days 2 to 3 of menstruation, followed by daily injections of 0.25 mg Cetrotide (Baxter Oncology GmbH, Frankfurt, Germany) once the leading follicle reached 14 mm and until the day of hCG injection.

The choice of protocol for ovarian stimulation was based on the patient's characteristics. When more than two leading follicles measured 18 mm or more, hCG was administered. After retrieval, oocytes were fertilized by standard insemination. Embryos were transferred on day 2 or 3. The luteal phase was supported by daily vaginal or intramuscular progesterone until 8 weeks after ET.

Data collection

Patient clinical parameters (patient age, day 3 follicle-stimulating hormone [FSH], LH, E_2 concentration, duration of infertility, type of protocol) were collected from our database. The outcomes of IVF were the primary outcomes. The secondary outcomes were the risk of adverse obstetric outcomes related to placentation.

The outcomes of IVF included the number of oocytes retrieved; the number of matured oocytes (MII oocytes, determined 16–18 h following retrieval for conventional IVF cycles); implantation rate and clinical pregnancy rate (documented intrauterine pregnancy with fetal heart activity).

Pregnancy outcomes were collected to include pre-term delivery (PT), birth weight, and the presence of PreE. The numbers of deliveries before 37 weeks (pre-term delivery), low birth weight (LBW) infants (defined as birth weight <2500 g), and PreE cases (defined as elevated systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg after 20 weeks gestation with the presence of proteinuria or clinical features) from 360 singleton IVF-ET pregnancies were calculated.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, Version 10 for Windows; SPSS, Inc., Chicago, IL, USA). The results are expressed as the mean ± standard deviation (SD), and categorical variables were expressed as the number of cases (n) and percentage of occurrence (%). Statistical analysis was performed using unpaired Student's t test and unpaired Student's t test and Wilcoxon rank-sum test to evaluate the continuous variables. The Chi-squared (χ^2) test was used to compare categorical data. All tests were conducted using a P value <0.05 to define statistical significance.

Odds ratios (ORs) were calculated. A receiving operator characteristic (ROC) curve was constructed to identify an E_2 threshold, with a corresponding area under the curve (AUC) calculation. Binary logistic regression analysis was used to assess whether the outcomes could be explained by age, parity, or other confounding variables.

Results

General information of patients

The mean age of all 1771 patients in our retrospective study was 32.69 (range, 22–40) years. A comparison of the demographic characteristics of the patients in the different groups did not reveal statistically significant differences in baseline LH, E_2, the duration of infertility, the primary infertility rate, or progesterone (P), LH, the endometrial thickness on the day of hCG injection. The number of embryos transferred was almost same in six groups.

The patients with $E_2 \leq 1000$ pg/mL had a higher average age (36.0 ± 5.1) years and a higher baseline FSH (9.0 ± 3.8) mIU/mL than the patients in the other groups. The patients with lower serum E_2 levels on the day of hCG administration were more likely to use a GnRH antagonist pituitary down-regulation stimulation protocol [Table 1]. Binary logistic regression analysis was performed to find that serum E_2 levels on hCG administration day appeared to be an independent risk factor for clinical pregnancy rate accounting for age, parity, duration of infertility, baseline FSH, LH, E_2, and P, LH, the endometrial thickness on the day of hCG injection and the number of embryos transferred [Table 2].

Ovulation-promoting and clinical results

Figure 2 shows the IVF outcomes of the patients according to their serum E_2 levels on the day of hCG administration. As shown in the figure, the number of retrieved oocytes (group 1: 4.1 ± 2.4; group 2: 7.0 ± 3.1; group 3: 10.0 ± 3.9; group 4: 11.1 ± 4.3; group 5: 12.9 ± 4.4; group 6: 12.5 ± 4.6), the number of retrieved MII oocytes (group 1: 3.5 ± 1.9; group 2: 5.5 ± 2.6; group 3: 8.0 ± 3.5; group 4: 9.0 ± 3.8; group 5: 11.0 ± 4.5; group 6: 10.5 ± 4.0), the implantation rate (group 1: 23.7% ± 3.4%; group 2: 31.3% ± 3.4%; group 3: 37.5% ± 3.8%; group 4: 38.1% ± 3.7%; group 5: 41.1% ± 3.8%; group 6: 37.7% ± 3.7%), and the clinical pregnancy rate gradually increased from group 1 to group 5 (group 1: 33.2% ± 4.7%; group 2: 47.7% ± 5.0%; group 3: 53.2% ± 5.0%; group 4: 55.2% ± 4.9%; group 5: 56.1% ± 4.8%; group 6: 55.5% ± 5.0%) but declined again in group 6. These parameters were highest in patients

Table 1: Comparison of baseline parameters between patients with different serum estradiol levels on the day of hCG administration.

Items	Group 1 ($E_2 \leq$ 1000 pg/mL)	Group 2 (E_2 = 1001–2000 pg/mL)	Group 3 (E_2 = 2001–3000 pg/mL)	Group 4 (E_2 = 3001–4000 pg/mL)	Group 5 (E_2 = 4001–5000 pg/mL)	Group 6 ($E_2 >$ 5000 pg/mL)
Number of patients	205	457	425	310	237	137
Age (years)	36.0 ± 5.1	33.5 ± 5.0*	31.9 ± 4.6*	31.8 ± 4.6*	30.8 ± 4.4*	32.0 ± 4.3*
BMI (kg/m^2), median (IQR)	22.8 (17.5)	21.3 (20.2)	21.9 (19.3)	21.2 (19.7)	21.7 (19.4)	22.7 (18.1)
Primary infertility, *n* (%)	109 (53.2)	253 (55.4)	235 (55.3)	195 (62.9)	153 (64.6)	81 (59.1)
Duration of infertility (years), median (IQR)	3 (5)	3 (4)	3 (4)	3 (4)	3 (4)	3 (4)
Basical FSH (mIU/mL)	9.0 ± 3.8	7.9 ± 2.5*	7.4 ± 2.2*	7.2 ± 2.0*	7.0 ± 1.9*	7.0 ± 1.7*
Basical LH (mIU/mL)	3.3 ± 1.7	3.5 ± 2.4	3.5 ± 2.1	3.7 ± 2.3	3.9 ± 2.7	3.7 ± 2.4
Basical E_2 (pg/mL)	39.1 ± 20.7	38.6 ± 24.9	36.5 ± 19.7	40.5 ± 28.5	39.3 ± 21.9	39.3 ± 24.3
Stimulation protocol						
GnRH agonist long protocol	123, 60.0%	371, 81.2%*	394, 92.7%*,†	294, 94.9%*,†	233, 98.4%*,†,‡,§	135, 98.5%*,†,‡,§
GnRH-antagonist protocol	82, 40%	86, 18.8%*	31, 7.3%*,†	16, 5.1%*,†	4, 1.7%*,†,‡,§	2, 1.5%*,†,‡,§
Dose of gonadotropin (IU)	2675.1 ± 1025.5	2750.0 ± 1019.8	2595.9 ± 925.1	2563.8 ± 931.4	2432.9 ± 847.6	2505.7 ± 842.5
hCG E_2 (pg/mL)	662.0 ± 205.1	1489.2 ± 283.6	2501.9 ± 304.3	3492.9 ± 296.2	4526.40 ± 294.0	5854.2 ± 786.5
hCG P (ng/mL)	0.64 ± 0.2	0.67 ± 0.2	0.66 ± 0.2	0.66 ± 0.2	0.67 ± 0.2	0.64 ± 0.1
hCG LH (mIU/mL)	2.15 ± 1.1	2.15 ± 1.3	2.22 ± 1.3	2.19 ± 1.3	2.23 ± 1.4	2.23 ± 1.4
The endometrial thickness on the day of hCG injection (mm)	10.0 ± 2.5	9.9 ± 2.6	10.0 ± 2.5	10.1 ± 2.6	9.9 ± 2.5	9.9 ± 2.5
Number of embryos transferred	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.4 ± 0.1	1.6 ± 0.2	1.5 ± 0.2

Values are expressed as mean ± standard deviation unless otherwise indicated. *$P < 0.05$ (*vs.* group 1). †$P < 0.05$ (*vs.* group 2). ‡$P < 0.05$ (*vs.* group 3). §$P < 0.05$ (*vs.* group 4). BMI: Body mass index; FSH: Follicle-stimulating hormone; GnRH: Gonadotrophin-releasing hormone; hCG: Human chorionic gonadotrophin; LH: Luteinizing hormone.

Table 2: Binary logistic regression analysis to account for confounding variables.

	Unadjusted OR (95% CI)						Adjusted OR (95% CI)					
					95% CI for Exp (B)						95% CI for Exp (B)	
Comparison	B	Wald	Sig.	Exp (B)	Lower	Upper	B	Wald	Sig.	Exp (B)	Lower	Upper
Age	−0.025	9.19	0.092	0.975	0.959	0.991	−0.021	2.724	0.099	0.979	0.954	1.004
Parity (0 *vs.* ≥1)	−0.121	1.976	0.16	0.886	0.749	1.049	−0.062	0.491	0.483	0.94	0.791	1.117
Duration of infertility	−0.012	0.715	0.398	0.988	0.961	1.016	−0.004	0.091	0.763	0.996	0.967	1.025
BMI	0.004	1.737	0.188	1.004	0.998	1.01	0.009	0.515	0.473	1.009	0.985	1.034
Basical FSH	−0.043	6.578	0.11	0.958	0.926	0.99	−0.028	2.525	0.112	0.972	0.939	1.007
Basical LH	0.035	3.393	0.065	1.035	0.998	1.074	0.026	0.257	0.612	1.026	0.928	1.134
Basical E_2	0.002	1.56	0.212	1.002	0.999	1.006	0.002	0.749	0.387	1.002	0.998	1.005
hCG E_2	0	30.431	0	1	1	1	0	13.326	0	1	1	1
hCG P	−0.147	0.438	0.508	0.863	0.558	1.335	0.661	1.368	0.242	1.938	0.64	5.87
hCG LH	0.036	1.227	0.268	1.037	0.973	1.105	−0.09	0.719	0.396	0.914	0.743	1.125
The endometrial thickness on the day of hCG injection	−0.014	0.667	0.414	0.986	0.954	1.019	−0.053	1.818	0.178	0.949	0.879	1.024
Number of embryos transferred	−0.183	0.488	0.485	0.832	0.498	1.392	−0.116	0.181	0.671	0.89	0.521	1.522

BMI: Body mass index; CI: Confidence interval; FSH: Follicle-stimulating hormone; GnRH: Gonadotrophin-releasing hormone; hCG: Human chorionic gonadotrophin; LH: Luteinizing hormone; OR: Odds ratio.

with serum E_2 levels between 4001 and 5000 pg/mL. All of the observed IVF outcomes were significantly lower in group 1 than in groups 2, 3, 4, 5, and 6:

1. The number of retrieved oocytes, $t = 13.096$, $P < 0.003$ (group 1 *vs.* group 2); $t = 23.307$, $P < 0.003$ (group 1 *vs.* group 3); $t = 23.086$, $P < 0.003$ (group 1 *vs.* group 4); $t = 26.376$, $P < 0.003$ (group 1 *vs.* group 5); $t = 19.636$, $P < 0.003$ (group 1 *vs.* group 6)
2. The number of retrieved MII oocytes, $t = 10.856$, $P < 0.003$ (group 1 *vs.* group 2); $t = 20.868$, $P < 0.003$ (group 1 *vs.* group 3); $t = 21.874$, $P < 0.003$ (group 1 *vs.* group 4); $t = 23.374$, $P < 0.003$ (group 1 *vs.* group 5); $t = 19.092$, $P < 0.003$ (group 1 *vs.* group 6)
3. The implantation rate, $\chi^2 = 12.179$, $P < 0.003$ (group 1 *vs.* group 2); $\chi^2 = 22.239$, $P < 0.003$ (group 1 *vs.* group 3); $\chi^2 = 23.993$, $P < 0.003$ (group 1 *vs.* group 4); $\chi^2 = 23.344$, $P < 0.003$ (group 1 *vs.*

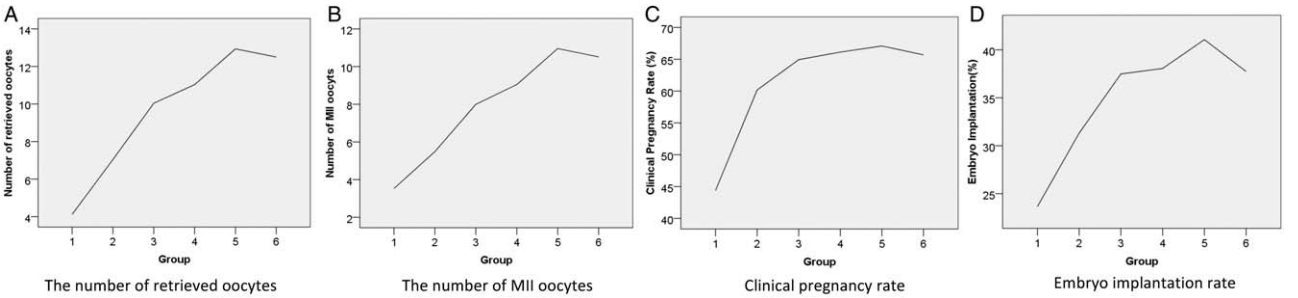

Figure 2: Relationship between IVF and pregnancy outcomes with increasing serum estradiol levels represented in different groups (Group 1: $E_2 \leq 1000$ pg/ml, Group 2: E_2 1001–2000 pg/ml, Group 3: E_2 2001–3000 pg/ml, Group 4: E_2 3001–4000 pg/ml, Group 5: E_2 4001–5000 pg/ml, Group 6: $E_2 > 5000$ pg/ml). The number of retrieved oocytes (A), the number of MII oocytes (B), embryo implantation rate (C) and clinical pregnancy rate (D) increased with increasing serum estradiol level until Group 5, with subsequent non-significant downward trend.

Table 3: Odds of term LBW with increasing E_2 levels.

	Unadjusted OR (95% CI)						Adjusted OR (95% CI)					
					95% CI for EXP (B)						95% CI for EXP (B)	
Referent group	B	Wals	Sig.	Exp (B)	Lower	Upper	B	Wals	Sig.	Exp (B)	Lower	Upper
$E_2 \leq 1000$ pg/mL ($n = 41$)												
E_2 1001–2000pg/mL ($n = 93$) *vs.* referent	0.587	0.267	0.605	1.798	0.195	16.600	0.846	0.533	0.465	2.331	0.240	22.605
E_2 2001–3000pg/mL ($n = 88$) *vs.* referent	0.879	0.625	0.429	2.410	0.272	21.315	1.156	0.997	0.318	3.179	0.328	30.767
E_2 3001–4000pg/mL ($n = 64$) *vs.* referent	1.743	2.601	0.107	5.714	0.687	47.515	1.997	3.170	0.075	7.365	0.818	66.356
E_2 4001–5000pg/mL ($n = 47$) *vs.* referent	2.249	4.349	0.037	9.474	1.145	78.388	2.442	4.692	0.03	11.496	1.262	104.748
$E_2 > 5000$ pg/mL ($n = 27$) *vs.* referent	2.824	6.631	0.010	16.842	1.963	144.498	3.267	7.599	0.006	26.223	2.571	267.515

CI: Confidence interval; LBW: Low birth weight; OR: Odds ratio.

group 5); $\chi^2 = 16.758$, $P < 0.003$ (group 1 *vs.* group 6)

4. The clinical pregnancy rate, $\chi^2 = 16.415$, $P < 0.003$ (group 1 *vs.* group 2); $\chi^2 = 28.074$, $P < 0.003$ (group 1 *vs.* group 3); $\chi^2 = 35.387$, $P < 0.003$ (group 1 *vs.* group 4); $\chi^2 = 37.025$, $P < 0.003$ (group 1 *vs.* group 5); $\chi^2 = 24.590$, $P < 0.003$ (group 1 *vs.* group 6)

Notably, there were no statistically significant differences in IVF outcomes between the other groups.

Of the 1771 patients, 360 had singleton pregnancies. The mean ± SD age and the median serum E_2 level on the day of the hCG trigger in the study cohort were 31.8 ± 2.91 years and 2559 pg/mL, respectively. The prevalence rates of PT, PreE, and LBW in these singleton deliveries were 14.1% (51), 5.3% (19), and 9.7% (35), respectively. The ORs for perinatal outcomes at various peak E_2 levels were calculated using the E_2 level of the ≤1000 pg/mL study cohort as the reference. The odds of LBW were higher in the top E_2 group than in the reference group, suggesting an E_2-dependent effect on LBW. The odds of LBW with E_2 levels >5000 pg/mL were 16.8 times higher than those with the E_2 levels of the reference group [Table 3]. Binary logistic regression analysis was performed to account for age, parity, ovarian stimulation protocol, and gonadotropin dose [Table 4]. As seen in Table 4, serum E_2 levels on hCG administration day appeared to be an independent risk factor for LBW. However, there were no differences in the odds of PT or PreE across the different E_2 groups.

Since higher peak serum E_2 levels were associated with a higher likelihood of LBW, we generated a ROC curve to determine an optimal average peak serum E_2 cutoff level for predicting LBW. ROC curve analysis demonstrated that a peak serum $E_2 > 3148$ pg/mL was associated with LBW with a sensitivity of 71.4%, a specificity of 68.3% and an AUC of 0.721 (SE, 0.051; 95% confidence interval, 0.624–0.824; $P < 0.01$) for LBW, and the serum peak E_2 cutoff value of 3148 pg/mL [Figure 3].

Discussion

Supraphysiologic E_2 levels are unavoidable during COH, and the effect of such supraphysiologic E_2 levels on the outcome of IVF-ET has remained controversial. The present study showed that the numbers of oocytes and MII oocytes received and the implantation and clinical pregnancy rates increased gradually as serum E_2 levels increased up to 5000 pg/mL, but these parameters began to decline at concentrations above 5000 pg/mL.

Table 4: Binary logistic regression analysis to account for confounding variables.

	Unadjusted OR (95% CI)						Adjusted OR (95% CI)					
					95% CI for Exp (B)						95% CI for Exp (B)	
Referent group	B	Wals	Sig.	Exp (B)	Lower	Upper	B	Wals	Sig.	Exp (B)	Lower	Upper
Term LBW ($n = 35$) E_2 level on the day of hCG trigger (<3297 *vs.* ≥3297 pg/mL)	1.679	19.113	<0.001	5.362	2.525	11.383	1.652	17.378	<0.001	5.22	2.4	11.351
Age (<35 *vs.* ≥35 years)	0.044	0.015	0.901	1.045	0.519	2.105	−1.222	2.714	0.099	0.295	0.069	1.261
Parity (0 *vs.* ≥1)	0.234	0.427	0.514	1.263	0.627	2.546	0.597	0.976	0.323	1.816	0.556	5.934
Ovarian stimulation protocol (GnRH-agonist *vs.* GnRH-antagonist protocol)	0.685	3.071	0.080	1.984	0.922	4.267	0.667	1.817	0.178	1.949	0.739	5.140
Gonadotropin dose (<2000 *vs.* ≥2000 IU)	0.43	1.44	0.23	1.538	0.761	3.105	0.527	0.809	0.369	1.693	0.537	5.337

CI: Confidence interval; GnRH: Gonadotrophin-releasing hormone; hCG: Human chorionic gonadotrophin; LBW: Low birth weight; OR: Odds ratio.

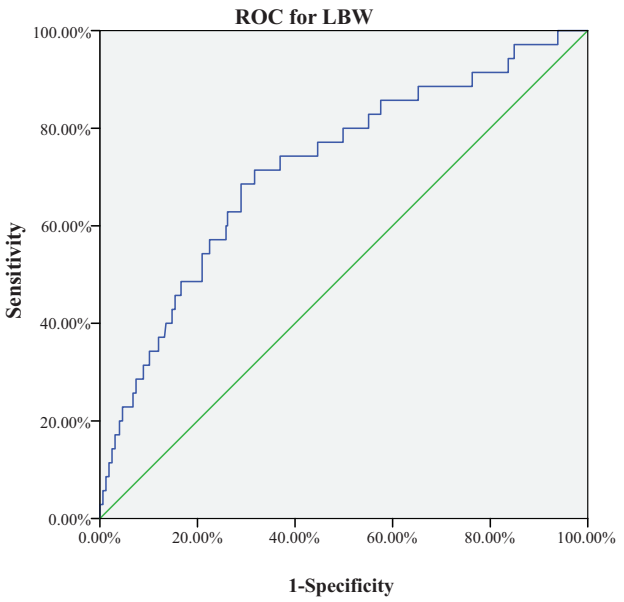

Figure 3: Receiver operating characteristic (ROC) curve evaluates the ability of peak E_2 measurements to predict low birth weight. Peak serum E_2 level of ≥3148 pg/mL is associated with low birth weight (LBW) with a sensitivity of 71.4%, a specificity of 68.3%, and an area under the curve of 0.721.

Patients in group 5 (serum E_2 levels 4001–5000 pg/mL, measured on the day of hCG administration) were consistently associated with optimal IVF outcomes compared with patients with other E_2 levels. Our data agrees with the data reported by Joo *et al*,[5] who showed that the implantation rate and clinical pregnancy rate increased steadily until the levels of peak serum E_2 reached 4000 pg/mL. In a previous study, Blazar *et al*[13] also reported that ongoing pregnancy rates increased with increasing E_2 until a plateau was reached at approximately 2500 pg/mL. Although the peak serum E_2 levels with the highest pregnancy rates were different between our study and the other studies, which may have been due to different hormonal analysis methods, all of these studies agree that extremely high serum peak E_2 levels are not always sufficient for good outcomes. First, endometrial receptivity is damaged because of a change in the ratio of E_2 to P.[14] Second, excessive E_2 levels directly affect the embryo, which may have a deleterious effect on embryonic implantation.[15] A previous study also suggested that milder ovarian stimulation produces fewer but higher quality oocytes.[16]

Estrogen and its receptor is a major factor whose effects improve endometrial reception for the priming of embryo implantation.[17] The current study attempted to better understand the relationship between peri-implantation E_2 levels and IVF outcomes by stratifying the patients into groups by E_2 level to determine whether a dose-response effect existed. We showed that the IVF outcomes (the number of retrieved oocytes and MII oocytes and the rates of implantation and clinical pregnancy) displayed an inverted U-shaped serum E_2 level response. This finding is in agreement with that of the Imudia study, which confirmed a typical biphasic response between the E_2 level on the day of hCG administration and the parameters of the IVF outcomes.[18] Thus, optimizing serum E_2 levels on the day of hCG administration might help improve IVF treatment outcomes, while insufficient or excess E_2 levels may have deleterious or no effects. In 2010, Joo *et al*[5] reported that there is an optimum range of serum E_2 levels that positively affects IVF outcome. Their results suggested 3000 to 4000 pg/mL for women <38 years and 2000 to 3000 pg/mL for women ≥38 years as optimal ranges of E_2 levels. The limited sample size in our study meant that we did not divide patients according to different ages, and our results showed that a serum E_2 level <1000 pg/mL or more than 5000 pg/mL had a negative effect on IVF outcomes, including the number of retrieved oocytes and MII oocytes and the rates of implantation and clinical pregnancy. Together, these results suggest that there is an optimal range of E_2 levels that affects IVF outcomes and that the maximum level of serum E_2 on the day of hCG administration might cause an unfavorable outcome because of disrupted endometrial receptivity.[4,13,18]

A previous report by Kalra *et al* has suggested a 1.73-fold higher probability of LBW at term for singletons from fresh autologous IVF than for singletons from frozen thawed cycles, based on 56,792 singletons.[19] The authors demonstrate that the ovarian stimulation-induced maternal environment appears to represent an independent mediator that contributes to the risk of LBW. In this study, we also found that the peri-implantation maternal hormonal milieu that is unique to COH during fresh ET cycles was associated with a higher risk of delivering LBW infants in singleton conceptions. The odds of delivering LBW infants were 16.8-fold greater in these patients than in patients with lower serum E_2 levels. The predictive accuracy of conditions associated with LBW using an E_2 level of 3148 pg/mL during fresh IVF cycles is modest, with a specificity of 68.3% and sensitivity of 71.4%. Pereira also highlighted the potential association by reporting that the odds of delivering LBW singletons were higher with E_2 levels >3069.2 pg/mL on the day of hCG administration than with E_2 levels below this cutoff.[20]

A compromised nutrient and oxygen supply from the placenta to the fetus is a major cause of LBW. Placental growth and maintenance are the results of circulating levels of E_2 at the time of implantation by the trophectoderm. In non-human primate pregnancies, E_2 plays a key role in optimal fetal growth, being critical for the morphologic and functional differentiation of the villous trophoblast.[21] Our data suggest that the serum E_2 level on the day of hCG trigger is associated with LBW. This result might reflect the abnormal remodeling of the spiral artery and trophoblast invasion, which has been shown in previous studies to be fully operational.[22,23]

Consistently, in the animal model, E_2 is the main hormone affecting endometrial growth and the modulation of uteroplacental blood flow, and theoretically, high E_2 concentrations are associated with abnormal placentation. In recent research, elevated E_2 levels impaired the expression of implantation-associated genes, which could lead to aberrant placentation.[8] Aberrant placentation may lead to a suboptimal blood supply in the growing placenta and subsequently cause stillbirth, small for gestational age (SGA), or PreE.[24,25] The present clinical studies also agree with earlier findings that high E_2 concentrations adversely affect perinatal outcomes. Farhi *et al* has found that the high E_2 concentration group of >10,000 pmol/L had significantly more complications related to abnormal placentation.[10] Another report demonstrates that patients undergoing COH for IVF with a peak serum E_2 level >4500 pg/mL on the day of hCG administration have a higher risk of developing disorders related to SGA infants and PreE.[26] However, we found no differences in the rates of pre-term birth for patients with higher or lower peak E_2 levels, which is consistent with data from Kalra *et al*.[19] Similarly, no association was identified between PreE and elevated E_2 levels. Although we did not find a statistically significant difference in pre-term delivery or PreE for the deliveries resulting from pregnancies achieved through fresh IVF cycles, the 14.1% and 5.3% overall rates, respectively were higher than the 5% and 3.9% rates, respectively, of all singleton live births in the literature.[27]

Therefore, larger prospective studies are needed to confirm these findings. Elevated E_2 levels might merely be a surrogate for another uncharacterized molecular marker.[9] The impact of the hyper-estrogenic milieu during COH on implantation and placentation is an active area of investigation.

This work analyzed 1771 patients to determine whether a dose-response effect of E_2 existed. The results show that there is an association between serum E_2 on the day of hCG administration and the odds of adverse pregnancy outcomes, such as LBW. The predictive accuracy of conditions associated with LBW using the E_2 level during fresh IVF cycles is modest, with a specificity of 68.3% and sensitivity of 71.4%. The findings of this study indicate that insufficient or excessive serum E_2 levels on the day of hCG administration will not contribute to IVF-ET outcomes and might even have negative effects. In the analysis of 360 singleton births, our data have shed light on a strong association between E_2 and LBW. Previous studies have shown that LBW is associated with adult cardiovascular disease, diabetes, and dyslipidemia.[28,29] ART providers should be aware of the possible adverse pregnancy outcomes associated with supraphysiologic E_2 levels on the hCG trigger day. However, we also acknowledge the weaknesses of the retrospective design of this study (we cannot exclude the possibility of unidentified confounding variables). This study from our hospital compared the IVF outcomes and obstetrical outcomes of women who underwent fresh ET, and the inherent limitation of a small sample size from a single institution is apparent. Additionally, given the known variability in the E_2 immunoassay among different centers, there is little comparability between the current study and those from other centers. A larger prospective study from other institutions will be needed to confirm our findings.

In conclusion, we find that serum E_2 levels on the day of hCG administration influences the IVF and pregnancy outcomes in a concentration-dependent manner. We observe that serum levels lower than 1000 pg/mL or above 3148 pg/mL might negatively affect clinical outcomes. Taking pregnancy complications into consideration, we should aim to optimize rather than maximize the serum E_2 level during IVF treatment.

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Conflicts of interest

None.

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