The effect of serum estrogen levels in early pregnancy on the rate of hypertensive disorders of pregnancy after frozen embryo transfer: a case control study

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Research Article

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Abstract

Background

Estrogen (E2) is a key regulator of trophoblast invasion and remodeling of uterine decidual spiral arterioles during placentation. However, there is little research on the effects of E2 in early pregnancy on placentation and subsequent preeclampsia. This study aims to evaluate the relationship between the rate of hypertensive disorders of pregnancy (HDP) and serum estrogen levels in early pregnancy following frozen embryo transfer (FET).

Methods

This retrospective study included all patients who conceived after FET with singleton delivery in the National Taiwan University Hospital from January 1, 2006, to December 31, 2020. A total of 729 patients were included in the analysis. The primary outcome was the rate of HDP.

Results

Serum estrogen levels at the fourth and fifth gestational weeks were significantly different between women with and without HDP after FET (393.4 ± 235.7 vs 479.0 ± 261.2 pg/ml, p=0.007 and 492.0 ± 274.5 vs 563.9 ± 263.0 pg/ml, p=0.015, respectively). Multivariable logistic regression with generalized estimating equations showed that each 100-pg/mL increase in estrogen levels increased the risk of HDP by 12% (p = 0.006) after adjustment for confounders. In a subanalysis stratified using FET protocol type (natural cycle, n = 225; hormone replacement cycle, n = 504), an increased rate of HDP (P-trend = 0.021 and 0.014, respectively) was observed with increasing estrogen level quartile at the fourth and fifth gestational weeks in the hormone replacement cycles, but not in the natural cycles.

Conclusions

Higher estrogen levels in early pregnancy were associated with a higher rate of HDP. For better obstetric outcomes, we may alter the estradiol supplementation in FET hormone replacement cycles or choose FET natural cycles. Our results indicate a link between estrogen levels in early pregnancy and the rate of HDP. Further studies are needed to investigate the underlying mechanism.

Trial registration

Retrospectively registered

Background

In comparison with fresh embryo transfer, the freeze-all strategy using frozen embryo transfer (FET) has demonstrated promising results, with comparable live birth rates in normoresponders or even better live birth rates in hyper-responders and a lower risk of ovarian hyperstimulation syndrome (1–5). In some

meta-analysis studies, the pregnancies resulting from FET cycles seem to have some better obstetric and perinatal outcomes, including antepartum hemorrhage, preterm birth, and perinatal mortality, compared to fresh embryo transfer (6, 7). The freeze-all strategy with FET was also applied for embryo accumulation in poor responders with advanced age, preimplantation genetic testing, and fertility preservation. Thus, the proportion of FET cycles has increased globally. In the United States, the percentage of the FET cycle has increased from 26.3% in 2011 to 39.1% in 2016 (from the Assisted Reproductive Technology National Summary Report). In Taiwan, the percentage of the FET cycle also has increased from 15.5% in 2011 to 78.0% in 2019.

When compared with that after fresh embryo transfer, pregnancy after FET was associated with a higher rate of pregnancy complications, particularly hypertensive disorders of pregnancy (HDP) (1, 2, 8). Preeclampsia and gestational hypertension, also known as HDP, are increasingly recognized as placental diseases (9, 10). That is, abnormal placentation early in the first trimester may lead to HDP.

Although estrogen (E2) is a key regulator of trophoblast invasion and remodeling of uterine decidual spiral arterioles during placentation, there is little research on the effects of E2 in early pregnancy on placentation and subsequent preeclampsia (11). Excessive E2 inhibited the proliferation of decidualized endometrial stromal cells in rodent models, resulting in less development of the spiral arteries, which was supposed to be one of the important factors of developing preeclampsia (12) Several pathophysiologic mechanisms of preeclampsia, including the imbalance of angiogenic factors, nitric oxide synthesis deficiency, and impaired maternal vascular endothelial functions, might also be modulated by E2 (13–15). Therefore, investigating the potential effect of E2 levels on the HDP rate will provide information for predicting HDP rate or improving obstetric outcomes when prescribing estradiol supplementation for luteal support or early pregnancy support in FET hormone replacement cycles (FET-HRC). The goal of this study was to assess the association between E2 levels in early pregnancy and the rate of HDP following FET.

Materials And Methods

Study populations

The data were provided from electronic medical records in the National Taiwan University Hospital (NTUH). Patients who both conceived following FET and delivered in the NTUH from January 1, 2006, to December 31, 2020, were included in the study. If the patient was included for more than once, the first pregnancy was included only in the analysis. The data on FET cycle regimens and obstetric complications were validated by following all patients' prenatal examinations at NTUH's outpatient department. Patients with chronic hypertension (HTN), diabetes mellitus (DM), and previous HDP and gestational DM were excluded from the study, as well as those with multiple pregnancies or who conceived after FET-stimulated/FET-modified natural cycles (Supplemental Fig. 1).

Among the FET cycles, there were two groups defined as follows:

- Hormone replacement cycles: oral estradiol (ESTRADE®, estradiol valerate, 2mg/tablet, Synmosa, Taipei, Taiwan) was used for endometrial preparation. The doses of estradiol were from 2 tablets to 3 tablets twice a day, depending on the thickness of endometrium and the experience of the physicians and continued to 8-9th gestational weeks. Oral, injectable, and transvaginal progesterone were used for endometrial maturation and luteal phase support.
- Natural cycles: patients did not take any medications before endogenous luteinizing hormone surge. Oral, injectable, and transvaginal progesterone were used for luteal phase support. There was no estradiol supplementation.

Definition of outcomes

HDP in this study includes gestational HTN and preeclampsia. Gestational HTN is defined as a systolic blood pressure of ≥ 140 mmHg or a diastolic blood pressure of ≥ 90 mmHg, or both, on two occasions at least 4 h apart after 20 weeks of gestation in a woman with a previously normal blood pressure. Preeclampsia was diagnosed when a previously normotensive patient developed new-onset HTN and proteinuria or new-onset HTN and significant end-organ dysfunction with or without proteinuria after 20 weeks of gestation or postpartum (16). All diagnoses were made by medical doctors.

Serum analysis, hormone measurement, and endometrial thickness assessment

Serum samples were analyzed using Immulite 2000 reproductive hormone assays (Diagnostic Product Corporation, Siemens, Los Angeles, CA, USA). The sensitivity was 15 pg/mL for estradiol. Intra- and inter-assay coefficients of variation were 6.7% and 9.7%, respectively, for estradiol. Serum E2 levels of all women who conceived from assisted reproductive technology (ART) in our institution were measured on the day “OV + 9” (midluteal phase, approximately third gestational week), “OV + 16” (approximately fourth gestational week), and “OV + 22” (approximately fifth gestational week). Day “OV” was defined as the day on which progesterone began to be used in FET-HRC and the day on which progesterone was measured approximately 1.0 ng/mL with dominant follicle disappeared in FET-NC.

Endometrial thickness was measured prior to the initiation of progesterone in FET-HRC as well as on the day of luteinizing hormone surge in FET-NC with transvaginal ultrasound (TOSHIBA Nemio XG [SSA-580A], Tokyo, Japan; probe PVM-651VT, 6MHz). An endometrial thickness measurement was ideally obtained with a transvaginal ultrasound probe placed perpendicular to the uterine midline and measuring in the sagittal plane. The endometrial thickness measurement should focus on the endometrial area that appears to be the thickest with the calipers placed at opposite points of the anterior and posterior endometrial-myometrial interfaces calculating the total double-layer thickness in millimeters.

Statistical analysis

Descriptive statistics are given by number (n) and percentages for categorical variables as well as by mean and standard deviation (SD) for continuous variables. The chi-squared test for dichotomous variables and the T-test and the Mann–Whitney test for continuous variables were used to assess differences between the groups. To account for any confounding factors between E2 levels and HDP rate,

multivariable logistic regression analysis was used. The probabilities of HDP were analyzed with logistic regression using generalized estimating equations (GEE), with within-individual correlations taken into account (controlling for repeated measurements of E2 levels at the different gestational weeks). E2 (pg/mL) multiplied by 0.01 was used in the analyses of logistic regression and GEE models. To further investigate the relationship between E2 levels and the rate of HDP in different FET protocols, the patients were categorized into four groups based on the quartiles of serum E2 levels. P-values of < 0.05 in Cochran–Armitage tests for trends were considered significant. All statistical analyses were performed using the SPSS software program, version 22.0 (SPSS, Chicago, IL, USA). The significant difference was defined as $P < 0.05$.

Results

Overall, 729 singleton deliveries were included in the study, including 677 pregnancies without HDP and 62 pregnancies with HDP. The overall rate of HDP was 8.5%. Table 1 shows the background characteristics of the non-HDP and HDP groups. The mean body mass index was higher in the HDP group than in the non-HDP group (24.6 ± 3.6 vs. 26.4 ± 4.6, p = 0.001). There were more nulliparous women in the HDP group (p = 0.001). These confounding factors were not included in the study due to the small number of cases with a history of smoking and alcohol consumption. With regard to the causes of infertility, the proportions of diminished ovarian reserve were higher in the HDP group than in the non-HDP group (p = 0.007). The category “others” included embryo freezing due to maternal disease, social or medical oocyte freezing, preimplantation genetic testing for monogenic diseases, chromosomal structural rearrangements, and aneuploidies. Concerning the protocols of FET, there were more HRC in the HDP group than in the non-HDP group (p = 0.004).

Table 1
Maternal and treatment characteristics of non-HDP and HDP

	Non-HDP (N = 667)	HDP (N = 62)	P-value
Maternal age, years	36.2 ± 4.1	37.0 ± 5.1	0.146
Maternal BMI (kg/m2)	24.6 ± 3.6	26.4 ± 4.6	0.001
Smoking	2	0	NA
Alcohol drinking	0	0	NA
Aspirin during pregnancy	65 (9.7%)	7 (11.3%)	0.696
Parity			0.001
Nulliparity	441 (66.1%)	54 (87.1%)	
Multiparity	226 (33.9%)	8 (12.9%)	
Gestational age at birth	38.2 ± 2.1	36.9 ± 2.3	0.001
Embryo stage at transfer			0.075
Cleavage	215 (32.2%)	23 (37.1%)	
Blastocyst	452 (67.8%)	39 (62.9%)	
Type of cryopreservation			0.840
Slow freezing	70 (10.5%)	6 (9.7%)	
Vitrification	597 (89.5%)	56 (90.3%)	
Cause of infertility			
Anovulation	105 (15.7%)	10 (16.1%)	0.936
Tubal factor	122 (18.3%)	11 (17.7%)	0.915
Male factor	266 (39.9%)	18 (29.0%)	0.094
Uterine factor	40 (6.0%)	4 (6.5%)	0.886
Endometriosis	37 (5.5%)	1 (1.6%)	0.182
DOR	155 (23.2%)	24 (38.7%)	0.007
Unexplained	73 (10.9%)	2 (3.2%)	0.056

*The number in the analysis was 697 due to missing estrogen data.

ART, assisted reproductive technology; BMI, body mass index; DOR, diminished ovarian reserve; FET, frozen embryo transfer; GW, gestational week; HDP, hypertensive disorders of pregnancy; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; NA, not applicable

	Non-HDP (N = 667)	HDP (N = 62)	P-value
Others	64 (9.6%)	12 (19.4%)	0.094
Fertilization methods			0.398
IVF	95 (14.2%)	5 (8.1%)	
ICSI	435 (65.2%)	43 (69.3%)	
Both IVF & ICSI	137 (20.6%)	14 (22.6%)	
Protocol of FET			0.004
Natural cycle	216 (32.4%)	9 (14.5%)	
Hormone replacement cycle	451 (67.6%)	53 (85.5%)	
Estrogen level (pg/ml)			
E2 levels at 3rd GW	337.9 ± 259.6	377.7 ± 210.0	0.261
E2 levels at 4th GW	393.4 ± 235.7	479.0 ± 261.2	0.007
E2 levels at 5th GW	492.0 ± 274.5	563.9 ± 263.0	0.015
Endometrial thickness (mm)	10.4 ± 2.5	10.5 ± 2.2	0.729
*The number in the analysis was 697 due to missing estrogen data.			
ART, assisted reproductive technology; BMI, body mass index; DOR, diminished ovarian reserve; FET, frozen embryo transfer; GW, gestational week; HDP, hypertensive disorders of pregnancy; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; NA, not applicable			

The E2 levels at the fourth (393.4 ± 235.7 vs. 479.0 ± 261.2 pg/mL, p = 0.007) and fifth gestational weeks (492.0 ± 274.5 vs. 563.9 ± 263.0 pg/mL, p = 0.015) were significantly different between the non-HDP and HDP groups, but not significantly different at the third gestational week (337.9 ± 259.6 vs 377.7 ± 210.0, p = 0.261).

Table 2 presents the analysis of the risk of HDP based on serum E2 levels. The multivariable logistic regression revealed that increases in E2 levels (per 100 pg/mL) at the fourth and fifth gestational weeks were associated with an increased rate of HDP (adjusted odds ratio (aOR), 1.15; 95% confidence interval [CI], 1.04–1.28 and aOR, 1.10; 95% CI, 1.01–1.20, respectively) after the adjustment for age, body mass index, parity, endometrial thickness, polycystic ovary syndrome, with or without aspirin use, stage at embryo transfer, type of cryopreservation, and fertilization methods. Multivariable logistic regression using GEE showed that the risk of HDP increased by 12% (p = 0.006) for each unit (100 pg/mL) increase in E2 levels at the fourth to fifth gestational weeks after the adjustment of confounders.

Table 2
Multivariable logistic regression and generalized estimating equations (GEEs) models analyses of the risk of HDP according to serum estrogen levels (pg/ml).

	Adjusted OR* (95%CI)	P-value
E2 levels at 3rd GW	1.08 (0.98–1.19)	0.140
E2 levels at 4th GW	1.15 (1.04–1.28)	0.006
E2 levels at 5th GW	1.10 (1.01–1.20)	0.035
E2 levels at 4-5th GW$	1.12 (1.03–1.21)	0.006
*$Adjust for age, BMI, parity, endometrial thickness, PCOS, aspirin use, embryo stage at transfer, type of cryopreservation, Fertilization methods		
$ GEE models have been used as they allow adjustment of within-individual correlation.		
E2 (pg/ml) multiplied by 0.01 were used in the analyses of logistic regression and GEE models.		
BMI, body mass index; CI, confidence interval; E2, estrogen; GW, gestational week; HDP, hypertensive disorders of pregnancy; OR, odds ratio; PCOS, polycystic ovary syndrome;		

Following subanalysis stratified using different FET protocols (Table 3 and Fig. 1), there was a statistically significant increase in the rate of HDP with increasing E2 level quartile at the fourth and fifth gestational weeks after FET-HRC (P-trend = 0.021 and 0.014, respectively), but there was no difference after FET-NC (P-trend = 0.583 and 0.246, respectively).

Table 3
Rate of HDP by E2 levels quartile after different FET protocols

	E2 levels 0-25th	E2 levels 25th – 50th	E2 levels 50th -75th	E2 levels 75th -100th	P trend
FET 4W HDP, n (%)	11 (6.1%)	12 (6.6%)	13 (7.1%)	26 (14.1%)	0.008
FET, 5W HDP, n (%)	13 (7.2%)	9 (5.0%)	18 (9.8%)	22 (12.0%)	0.038
FET-NC, 4W HDP, n (%)	3 (5.7%)	2 (3.5%)	2 (3.5%)	2 (3.4%)	0.583
FET-NC, 5W HDP, n (%)	2 (3.8%)	4 (7.0%)	3 (5.3%)	0 (0%)	0.246
FET-HRC, 4W HDP, n (%)	9 (7.3%)	10 (7.9%)	14 (11.1%)	20 (15.6%)	0.021
FET-HRC, 5W HDP, n (%)	9 (7.3%)	8 (6.3%)	17 (13.6%)	19 (14.8%)	0.014

E2, estrogen; FET-HRC, frozen embryo transfer hormone replacement cycles; FET-NC, frozen embryo transfer natural cycles; HDP, hypertensive disorders of pregnancy

Discussion

It was observed in this retrospective cohort study that increasing E2 levels in early pregnancy was associated with higher HDP rates. The findings of this study support the concept that E2 levels may have an effect on the placentation and subsequent obstetric outcomes in animal models. To our knowledge, this is the first study that investigated the associations between the serum E2 levels in early pregnancy and the risk of HDP after FET.

The role of E2 in regulating placentation was controversial. According to an *in vitro* trophoblast model, human chorionic gonadotropin and progesterone, but not E2, play direct roles in controlling trophoblast movement during vascular remodeling in early pregnancy (17). However, the angiogenic markers vascular endothelial growth factor, placental growth factor, and endothelial nitric oxide synthase, which regulate angiogenic processes, may be affected by E2 (15, 18, 19). It has been hypothesized that low levels of estradiol may result in insufficient trophoblast development and angiogenesis (20). Prematurely increasing E2 in early pregnancy, however, suppressed extravillous cytotrophoblast invasion and remodeling of the uterine spiral arteries in animal models, based on several studies (21–23).

According to one small case–control study, estradiol levels in early pregnancy (median 9.8 weeks, range 5.3–15.3 weeks) were increased in preeclamptic pregnancies compared with control pregnancies (24). Furthermore, several retrospective studies revealed that elevated peak serum E2 levels during controlled ovarian hyperstimulation were associated with higher risk of preeclampsia and abnormal implantation of the placenta after fresh embryo transfer (25, 26). These observations are consistent with a previous animal study in which the authors discovered that higher E2 levels had a detrimental effect on the arterial modeling of the uterus during early pregnancy (27).

Taken together, E2 is critical to placentation. It has been hypothesized that low but adequate levels of E2 exhibited during early pregnancy are required to allow normal progression of trophoblast vascular invasion and that the elevation of E2 level later in the second trimester has a physiological role in suppressing further arterial trophoblast invasion (23). Our findings support a part of this hypothesis: excessive E2 in early pregnancy might have negative effects on placentation. The effects of E2 could be complicated, and any of these effects on placentation are likely dependent on the levels and timing.

In this study, the serum E2 levels did not alter the risk of HDP in the FET-NC group. This could be due to the relatively small sample size and lower mean E2 levels in NC, compared with HRC (Supplementary Table 1). That is, there may be fewer patients with excessive E2 levels in early pregnancy following FET-NC than following FET-HRC.

Strengths And Limitations

All cases were followed at our institution, and the medications prescribed throughout ART cycles and pregnancies were documented. Thus, we could adjust some confounding factors, such as aspirin prescribed during pregnancy; prior history of HDP or GDM; and history of PCOS, DM, or HTN. Estradiol valerate was only prescribed as estradiol supplementation for all FET-HRC during endometrial preparation and at early gestational weeks, reducing the risk of bias when assessing serum E2 levels.

This study had some limitations. This was a retrospective study that might contribute to selection bias. All the confounding factors were not adjusted. The risks of bias and residual confounding factors were always a concern, even after multivariate logistic regression analysis. Although the results were statistically significant, caution should be exercised in their interpretation because HDP is a complex multifactorial outcome that cannot be explained using a single factor. More research is needed to understand the underlying mechanism and evaluate what levels of E2 in early pregnancy are supposed to be considered excessive.

Conclusion

In conclusion, this is the first study to demonstrate an association between the serum E2 levels in early pregnancy and the risk of HDP after FET.

We found that higher E2 levels in early pregnancy were linked to an increased rate of HDP after FET. Our findings suggest a need for caution when prescribing estradiol supplementation in early pregnancy after FET-HRC. That is, adequately but not excessively prescribing estradiol for luteal phase support or early pregnancy support in FET-HRC may reduce the risk of HDP. However, further prospective studies are needed to determine the association between the serum E2 levels in early pregnancy and the risk of HDP.

Abbreviations

aOR : adjusted odds ratio

DM : diabetes mellitus

E2 : estrogen

FET : frozen embryo transfer

FET-HRC : frozen embryo transfer hormone replacement cycles

GEE : generalized estimating equations

HDP : hypertensive disorders of pregnancy

HTN : hypertension

NTUH : National Taiwan University Hospital

SD : standard deviation

Declarations

Ethical approval and consent to participate

The study was approved by the ethics committees at the National Taiwan University Hospital (202109103RIND). This study was carried out in accordance with the Declaration of Helsinki. Since this is a retrospective investigation, patients were not asked to participate in this study.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of their containing information that could compromise the privacy of patients.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y.C.H. performed the statistical analysis and wrote the manuscript; T.C.K., I.J.Y., P.K.Y., K.H.C., M.J.C., J.H.Y., and S.U.C. reviewed and edited the manuscript; M.J.C. and S.U.C. designed the research. All authors read and approved the final manuscript.

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Figures

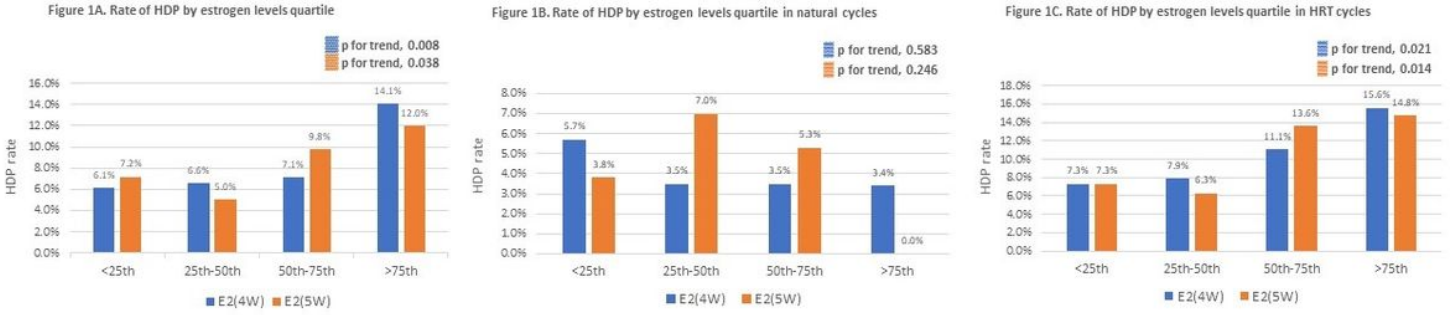

Figure 1

(A) Rate of HDP by estrogen levels quartile (B) Rate of HDP by estrogen levels quartile in natural cycles (C) Rate of HDP by estrogen levels quartile in HRT cycles

There was a statistically significant increased in the rate of HDP with increasing E2 levels quartile at the fourth and fifth gestational weeks after FET hormone replacement cycles (P-trend = 0.021 and 0.014, respectively) but no difference was observed after FET natural cycles (P-trend = 0.583 and 0.246, respectively).

E2, estrogen; FET, frozen embryo transfer; HDP, hypertensive disorders of pregnancy; HRT, hormone replacement

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigure1..pptx