Journal of Developmental Origins of Health and Disease

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Original Article

Cite this article: Duan C-C, Li C, He Y-C, Xu J-J, Shi C-Y, Hu H-T, Su Y-F, Chen L, Tan Y-J, Liu Z-W, Sheng J-Z, Fraser WD, Wu Y-T, and Huang H-F (2020) Oocyte exposure to supraphysiological estradiol during ovarian stimulation increased the risk of adverse perinatal outcomes after frozen-thawed embryo transfer: a retrospective cohort study. *Journal of Developmental Origins of Health and Disease* **11**: 392–402. doi: 10.1017/S2040174419000679

Received: 1 July 2019
Revised: 17 August 2019
Accepted: 19 September 2019
First published online: 4 November 2019

Keywords:
Frozen embryo transfer; high estradiol; pregnancy outcomes; preterm; small for gestational age

Address for correspondence:
Yan-Ting Wu and He-Feng Huang, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, No. 910, Rd. Hengshan, Shanghai 200030, China. Emails: yanting_wu@163.com and Huanghefg@sjtu.edu.cn

†Chen-Chi Duan and Cheng Li should be considered similar in author order.

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Oocyte exposure to supraphysiological estradiol during ovarian stimulation increased the risk of adverse perinatal outcomes after frozen-thawed embryo transfer: a retrospective cohort study

Chen-Chi Duan[1,2,3,†], Cheng Li[1,2,3,†], Yi-Chen He[1,2,3], Jing-Jing Xu[1,2,3], Chao-Yi Shi[1,2,3], Hong-Tao Hu[1,2,3], Yun-Fei Su[1,2,3], Lei Chen[1], Ya-Jing Tan[1,2,3], Zhi-Wei Liu[1,2,3], Jian-Zhong Sheng[4], William D. Fraser[5], Yan-Ting Wu[1,2,3] and He-Feng Huang[1,2,3]

[1]International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200030, China, [2]Shanghai Key Laboratory of Embryo Original Diseases, Shanghai 200030, China, [3]Institute of Embryo-Fetal Original Adult Diseases Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China, [4]Department of Pathology and Pathophysiology, School of Medicine, Zhejiang University, Zhejiang, China and [5]Centre de recherche du Centre Hospitalier Universitaire de Sherbrooke (CRCHUS) and Department of Obstetrics and Gynecology, University of Sherbrooke, Sherbrooke, QC, Canada

Abstract

Maternal supraphysiological estradiol (E2) environment during pregnancy leads to adverse perinatal outcomes. However, the influence of oocyte exposure to high E2 levels on perinatal outcomes remains unknown. Thus, a retrospective cohort study was conducted to explore the effect of high E2 level induced by controlled ovarian stimulation (COH) on further outcomes after frozen embryo transfer (FET). The study included all FET cycles ($n = 10{,}581$) between 2014 and 2017. All cycles were categorized into three groups according to the E2 level on the day of the human Chorionic Gonadotropin trigger. Odds ratios (ORs) and their confidence intervals (CIs) were calculated to evaluate the association between E2 level during COH and pregnancy outcomes and subsequent neonatal outcomes. From our findings, higher E2 level was associated with lower percentage of chemical pregnancy, clinical pregnancy, ongoing pregnancy, and live birth as well as increased frequency of early miscarriage. Preterm births were more common among singletons in women with higher E2 level during COH ($aOR_1 = 1.93$, 95% CI: 1.22–3.06; $aOR_2 = 2.05$, 95% CI: 1.33–3.06). Incidence of small for gestational age (SGA) was more common in both singletons ($aOR_1 = 2.01$, 95% CI: 1.30–3.11; $aOR_2 = 2.51$, 95% CI: 1.69–3.74) and multiples ($aOR_1 = 1.58$, 95% CI: 1.03–2.45; $aOR_2 = 1.99$, 95% CI: 1.05–3.84) among women with relatively higher E2 level. No association was found between high E2 level during COH and the percentage of macrosomia or large for gestational age. In summary, oocyte exposure to high E2 level during COH should be brought to our attention, since the pregnancy rate decreasing and the risk of preterm birth and SGA increasing following FET.

Introduction

In vitro fertilization and embryo transfer (IVF-ET) has been increasingly used since it was first reported in 1978, and almost 8 million babies had been born after IVF-ET worldwide every year.[1] However, studies revealed higher risks of low birthweight (LBW) and small for gestational age (SGA), preterm delivery, intrauterine growth restriction, placental abnormality, gestational diabetes, and gestational hypertension in IVF-ET compared with spontaneous conception.[2–5] In this circumstance, awareness and concerns related to the adverse perinatal outcomes associated with IVF-ET have become increasingly prominent.

Controlled ovarian stimulation (COH) has taken an important role in the procedure of IVF-ET to obtain a higher number of oocytes.[6] Notably, maternal serum estradiol (E2) levels induced by COH were 10–20 times higher than normal.[7] Although higher E2 level has been regarded as indicating a better response to ovarian stimulation, exposure to supraphysiological E2 during pregnancy might lead to negative perinatal and neonatal outcomes. According to several studies, high E2 level induced by COH might sustain for more than 8 weeks following fresh embryo transfer, and lead to adverse effects on endometrial receptivity and intrauterine fetal growth.[8,9] Despite the fact that E2 in ovarian follicular fluid from regularly menstrauting women was found up to 200- to 1000-fold higher than in serum,[10] few studies focus on the impact of oocyte exposure to supraphysiological E2 during COH on further pregnancy outcomes.

Frozen-thawed embryo transfer (FET) enables women who have undergone COH to recover their initial hormone levels before embryo transfer. Although FET appears to partially protect from the adverse effects of high maternal E2 during early pregnancy, a 10-year national cohort from the Nordic countries indicated that the risk of LBW or SGA after FET was still higher than that after spontaneous conception.[11,12] Thus, it is of great interest to investigate whether high E2 level induced by COH affects the oocyte maturation and lead to adverse perinatal outcomes following FET.

With a prominent lower level of maternal E2 during implantation and early pregnancy, FET cycles become an ideal model to investigate the potential associations between oocyte exposure to markedly increased E2 levels during COH and both short- and long-term outcomes including pregnancy rates, adverse obstetric complications, and perinatal outcomes.

Methods

Study design and participants

This retrospective cohort study included all FET cycles from January 2014 to September 2017 at International Peace Maternity and Child Health Hospital (IPMCHH). Women of advanced age (over 40 years) undergoing FET were excluded due to their increased probability of abnormal basal hormone levels with reduced ovarian reserve. Women who received donated oocytes or sperm or those who underwent preimplantation genetic testing were excluded. Mixed transfers with two embryos retrieved from different oocyte retrieval cycles were also excluded. All cycles were then categorized into three groups according to the maternal serum E2 level on the day of the human chorionic gonadotropin (hCG) trigger (Group I: <10,000 pmol/l; Group II: 10,000–15,000 pmol/l; Group III: >15,000 pmol/l). The cutoff value of 10,000 pmol/l was selected based on our previous observation that E2 values above this level had adverse effects on the offspring.[8] The cutoff value of 15,000 pmol/l was selected based on the documented increased risk of a subsequent OHSS among patients with an E2 level over 15,000 pmol/l.[13,14]

Ethical approval for the study was obtained from the Institutional Review Board of IPMCHH (GKLW-2016-21). Written informed consent was obtained from all participants before inclusion.

The datasets used and/or analyzed during the current study are available from ResMan Research Manager of Chinese Clinical Trial Registry with the agreement of corresponding authors on reasonable request.

ART Procedures

The process of IVF was conducted according to our standard protocols, including ovarian stimulation, oocyte retrieval, and insemination by either conventional IVF or intracytoplasmic sperm injection. FET was performed following endometrial preparation by natural monitoring, an ovarian stimulation cycle, or hormone replacement therapy. Serum hormone levels, including E2, progesterone (P4), and luteinizing hormone (LH) were detected in the hospital clinical chemistry laboratory on the day of hCG trigger and before embryo transfer separately. Endometrial thickness before FET was measured by highly trained sonographers via transvaginal ultrasound.

Patient data regarding the ART procedure including oocyte retrieval and embryo transfer were collected from the patient's hospital records. Information that was documented included the type of COH protocol (gonadotropin-releasing hormone [GnRH]-agonist protocol, GnRH-antagonist protocol, the microflare protocol, natural cycles or others), the type of insemination (IVF or intracytoplasmic sperm injection), number of oocytes retrieved (≤10, 11–20 or >20), duration of embryo cryopreservation (<3, 3–6, or >6 months), the type of endometrium preparation (natural cycle, hormone replace therapy cycle, or ovarian stimulation cycle), the day of embryo transfer (day 2, day 3, or day 5), and the number of embryos transferred (1 or 2). Pregnancy outcomes and relevant obstetric outcomes were followed up as previously described.[15]

Outcomes measurements and variable specification

Participants were interviewed in person to obtain information on sociodemographic characteristics and reproductive history. The height and weight were measured, and body mass index (BMI) was calculated before interview. Queries to which the participants did not reply were considered as missing data.

Pregnancy outcome measures following FET were assessed by the serum β-hCG level and ultrasound scans. Additionally, adverse outcomes including ectopic pregnancy, early miscarriage before 12 gestational weeks, stillbirth, and pregnancy termination due to fetal defects were also assessed.

Obstetric complications and neonatal outcomes were abstracted from the participants' health records, including gestational hypertensive disorder (gestational hypertension, mild preeclampsia, or wild preeclampsia), gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, meconium staining of the amniotic fluid, vanishing twin, mode of delivery (vaginal or caesarean section), birthweight, and gender of neonates. Large for gestational age (LGA), SGA, and appropriate for gestational age were defined according to a global reference for fetal weight and birthweight of our population for a given gestational age and sex.[16]

Statistical analysis

Continuous variables with a normal distribution are represented as the means ± standard deviations, and differences among groups were tested by one-way analysis of variance. Continuous variables with a skewed distribution are represented as medians and interquartile ranges, and differences were tested by the Kruskal–Wallis test. Categorical outcome variables are represented as frequencies with proportions, and differences between each group were modeled using multinomial logistic regression, differences for trend were detected by the Cochran–Mantel–Haenszel χ^2 test.

Obstetric and neonatal outcomes were stratified according to singleton or multiple deliveries. The odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using logistic regression, to estimate the relationships between supraphysiological E2 exposure during COH and each outcome following FET. To analyze the obstetric complications and neonatal outcomes of singletons, multinomial logistic regression analyses were also performed to adjust ORs for potential confounding factors. When analyzing the neonatal outcomes of multiples, ORs and 95%CIs were obtained using multilevel logistic regression and adjusted for the corresponding confounding factors, according to Carlin *et al.*[17]

SAS software version 9.3 (SAS Institute, Inc, Cary, NC) was used to perform all statistical analyses. All p values were calculated using two-sided tests. Differences between values were considered statistically significant at a p value of less than 0.05. For multiple comparison, p values of less than 0.017 were considered to be statistically different.

https://doi.org/10.1017/S2040174419000679 Published online by Cambridge University Press

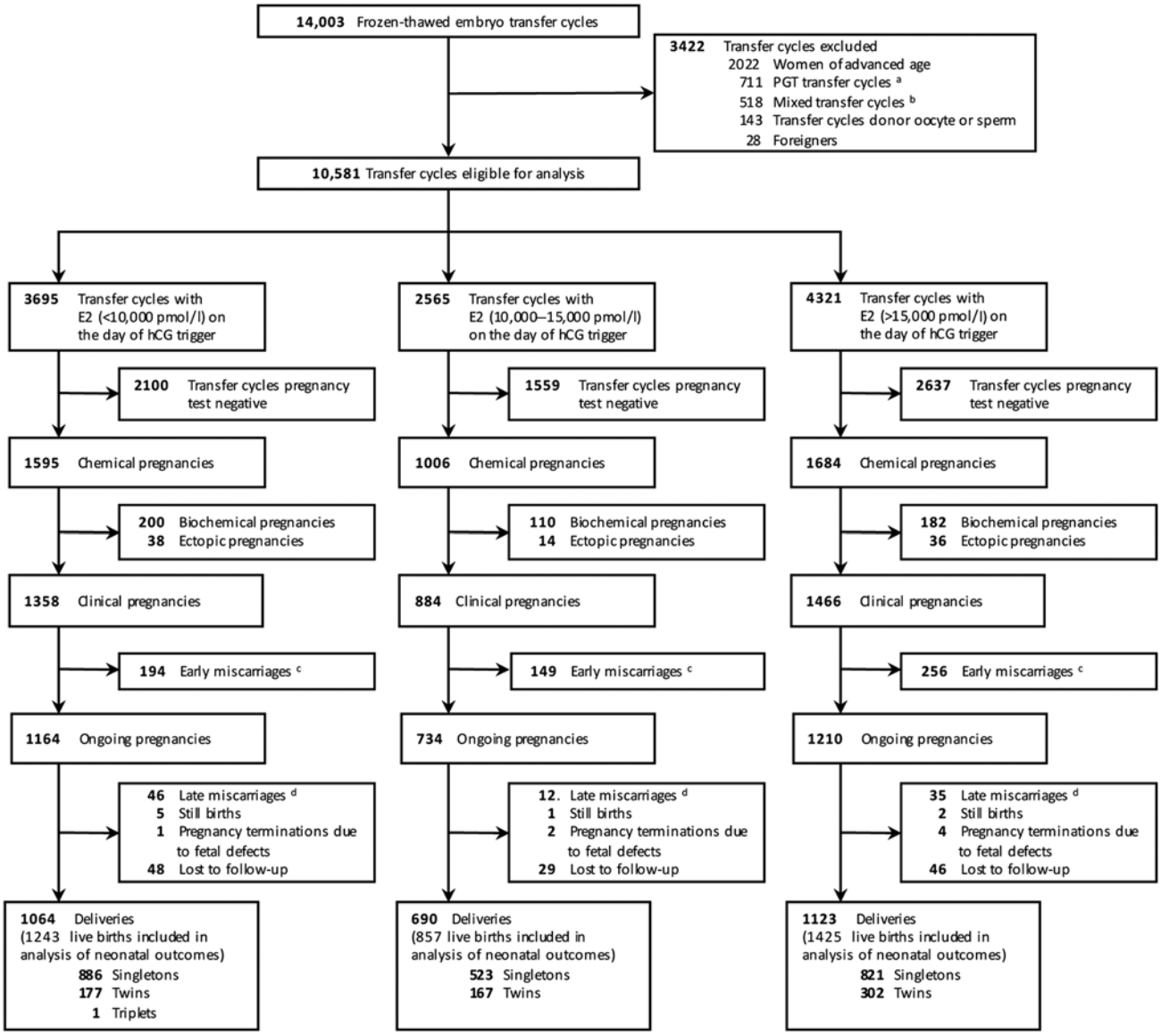

Fig. 1. Study flow chart. (a) PGT, preimplantation genetic testing. (b) Mixed transfer cycle was defined as transferring two embryos from different oocyte retrieval cycles. (c) Early miscarriage was defined as spontaneous loss of pregnancy before 12 gestational weeks. (d) Late miscarriage was defined as pregnancy loss between 12 and 28 gestational weeks.

Results

The flow of the study participants was shown in Fig. 1. A total of 10,581 FET cycles met the eligibility criteria and were included in the analysis (3695 cycles in Group I, 2565 cycles in Group II, and 4321 cycles in Group III). Women with live-born babies (2877 live births with 2230 singletons and 1295 multiples) were included in the analysis of obstetric complications and neonatal outcomes, with 123 participants lost to follow-up.

Reproduction history including maternal age at oocyte retrieval and at embryo transfer, pregestational BMI, duration of infertility, primary infertility, and causes of infertility were similar among three groups (see Table 1). However, the distribution of COH protocols administered was different among groups ($p < 0.001$). Patients with higher E2 level on the day of hCG trigger were more likely to have experienced GnRH-agonist protocol, while those with lower E2 level were more likely to have experienced microflare protocol. Furthermore, the number of oocytes retrieved tended to increase as E2 levels increased on the day of hCG trigger ($p < 0.001$). Notably, serum levels of P4 and LH showed no differences on the day of hCG trigger among groups, and no differences were found in endometrial thickness and hormone levels before FET among three groups either.

Table 2 shows the pregnancy outcomes per transfer cycle of the three groups. After confounding factors were adjusted, patients with higher E2 level on the day of hCG trigger showed lower percentage of chemical pregnancies (Group I: 43.17%, Group II: 39.22%, Group III: 38.97%, $p_{trend} < 0.001$), clinical pregnancies (Group I: 36.75%, Group II: 34.46%, Group III: 33.93%, $p_{trend} = 0.009$), ongoing pregnancies (Group I: 31.50%, Group II: 28.62%, Group III: 28.00%, $p_{trend} < 0.001$), and live births (Group I: 28.80%, Group II: 26.90%, Group III: 25.99%, $p_{trend} = 0.005$) per transfer cycle than those with lower E2 levels during COH. In addition, the frequency of early pregnancy loss increased as E2 levels increased on the day of hCG trigger (Group I: 14.29%, Group II: 16.86%, Group III: 17.46%, $p_{trend} = 0.023$). No differences were found in ectopic pregnancy rate ($p_{trend} = 0.374$), still birth rate ($p_{trend} = 0.190$), or pregnancy

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Table 1. Baseline characteristics of all FET cycles

	E2 level on the day of hCG trigger (pmol/l)			
	Group I: <10,000	Group II: 10,000–15,000	Group III: >15,000	
	(*N* = 3695)	(*N* = 2565)	(*N* = 4321)	
	No. (%)	No. (%)	No. (%)	*P*
History of reproduction				
Age at oocyte retrieval (Mean ± SD, years)	30.73 ± 4.02	30.72 ± 3.81	30.59 ± 3.74	0.077
Age at embryo transfer (Mean ± SD, years)	31.14 ± 4.13	31.31 ± 3.78	31.12 ± 3.68	0.110
Pregestational BMI (Mean ± SD, kg/m^2)	21.85 ± 3.63	21.98 ± 3.03	21.83 ± 3.19	0.067
Duration of infertility				
1–2	1430 (38.70)	968 (37.74)	1725 (39.92)	0.303
3–4	1259 (34.07)	873 (34.04)	1479 (34.23)	
≥5	1006 (27.23)	724 (28.23)	1117 (25.85)	
Primary infertility				
No	2181 (59.03)	1623 (63.27)	2527 (58.48)	0.514
Yes	1514 (40.97)	942 (36.73)	1794 (41.52)	
Causes of infertility				
Tubal infertility	1384 (37.46)	964 (37.58)	1645 (38.07)	0.215
Anovulatory	216 (5.85)	144 (5.61)	349 (8.08)	
Endometriosis	136 (3.68)	56 (2.18)	62 (1.43)	
Male factor infertility	145 (3.92)	92 (3.59)	215 (4.98)	
Unexplained infertility	824 (22.30)	630 (26.60)	914 (21.15)	
Combined*	990 (26.79)	678 (26.47)	1136 (26.29)	
Characteristics of oocytes retrieval cycle				
COH protocol				
GnRH-agonist regimen	801 (21.68)	734 (28.62)	1485 (34.37)	<0.001
GnRH-antagonist regimen	2219 (60.05)	1709 (66.63)	2657 (61.49)	
Microflare protocol	534 (14.45)	86 (3.35)	134 (3.10)	
Others	141 (3.82)	36 (1.40)	45 (1.04)	
Type of insemination				
IVF	2521 (68.23)	1742 (67.91)	2948 (68.22)	0.993
ICSI	1174 (31.77)	823 (32.09)	1373 (31.78)	
Number of oocytes retrieved				
Number, median (IQR)	8 (4–12)	12 (9–16)	16 (11–21)	<0.001
≤10	2486 (67.28)	959 (37.39)	851 (19.69)	<0.001
11–20	996 (26.96)	1335 (52.05)	2202 (50.96)	
>20	213 (5.76)	271 (10.57)	1268 (29.35)	
Hormone level on the day of hCG trigger				
P4, Median (IQR) (nmol/l)	3.0 (1.9–4.1)	2.9 (2.4–3.9)	2.9 (2.4–4.1)	0.530
LH, Median (IQR) (IU/l)	1.8 (1.1–3.6)	1.9 (1.1–3.2)	1.9 (1.1–3.2)	0.350
Characteristics of FET cycle				
Type of endometrium preparation				
Natural cycle	1603 (43.38)	1242 (48.46)	1883 (43.58)	0.269
OS cycle	326 (8.82)	269 (10.49)	483 (11.18)	
HRT cycle	1766 (47.79)	1053 (41.05)	1955 (45.24)	
Day of embryo transfer				
Day 3	2234 (60.46)	1508 (58.79)	2575 (59.59)	0.299
Day 4	975 (26.39)	682 (26.59)	1124 (26.01)	
Day 5	486 (13.15)	375 (14.62)	622 (14.39)	

(*Continued*)

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Table 1. (*Continued*)

	E2 level on the day of hCG trigger (pmol/l)			
	Group I: <10,000 (*N* = 3695)	Group II: 10,000–15,000 (*N* = 2565)	Group III: >15,000 (*N* = 4321)	
	No. (%)	No. (%)	No. (%)	*P*
Number of embryo transferred				
1	1022 (27.66)	547 (21.33)	1003 (23.21)	<0.001
2	2673 (72.34)	2018 (78.67)	3318 (76.79)	
Endometrial thickness (Mean ± SD, mm)	9.43 ± 1.85	9.48 ± 1.56	9.40 ± 1.53	0.107
Hormone level before embryo transfer				
E2, Median (IQR) (×10^3 pmol/l)	2.52 (1.75–3.98)	2.66 (1.43–4.61)	2.61 (1.41–4.13)	0.215
P4, Median (IQR) (nmol/l)	2.9 (1.7–4.6)	2.9 (1.6–4.6)	3.0 (1.7–4.7)	0.264
LH, Median (IQR) (IU/l)	9.8 (5.6–14.4)	9.7 (5.5–13.9)	9.7 (5.1–14.0)	0.050

FET, frozen embryo transfer; BMI, body mass index; COH, controlled ovarian stimulation; ART, assisted reproductive technology; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; OS, ovarian stimulation; HRT, hormonal replace therapy; E2, estradiol; P4, progesterone; LH, luteinizing hormone.
*Combined was defined as two or more infertile causes mentioned above.

Table 2. Pregnancy outcomes following transferring frozen-thawed embryos with different E2 exposure

	E2 level on the day of hCG trigger (pmol/l)					
	Group I: <10,000 (*N* = 3695)	Group II: 10,000–15,000 (*N* = 2565)	Group III: >15,000 (*N* = 4321)			
	No. (%)	No. (%)	No. (%)	P_1[a]	P_2[a]	$P_{for\ trend}$[b]
No. of chemical pregnancy (%)[c]	1595 (43.17)	1006 (39.22)	1684 (38.97)	<0.001	<0.001	<0.001
No. of clinical pregnancy (%)[d]	1358 (36.75)	884 (34.46)	1466 (33.93)	<0.001	<0.001	0.009
No. of ongoing pregnancy (%)[e]	1164 (31.50)	734 (28.62)	1210 (28.00)	<0.001	<0.001	<0.001
No. of live birth (% per transferred cycle)[f]	1064 (28.80)	690 (26.90)	1123 (25.99)	<0.001	<0.001	0.005
No. of ectopic pregnancy (%)	38 (1.03)	14 (0.55)	36 (0.83)	0.061	0.594	0.374
No. of early miscarriage (% per clinical pregnancy)	194 (14.29)	149 (16.86)	256 (17.46)	0.009	<0.001	0.023
No. of stillbirth (% per clinical pregnancy)	5 (0.37)	1 (0.11)	2 (0.14)	0.296	0.293	0.190
No. of pregnancy termination due to fetal defect (% per clinical pregnancy)	1 (0.07)	2 (0.23)	4 (0.27)	0.588	0.136	0.225

[a]*P*-values were calculated using multinomial logistic regression, and adjusted for age at oocyte retrieval, pregestational BMI, COH protocol, number of previous ART, number of oocytes retrieved, and number of embryo transferred. P_1 for comparisons between Group II and Group I, and p_2 for comparisons between Group III and Group I.
[b]$P_{for\ trend}$ was calculated using Cochran–Mantel–Haenszel test, and adjusted for age at oocyte retrieval, pregestational BMI, COH protocol, number of oocytes retrieved, and number of embryo transferred.
[c]Chemical pregnancy was defined as an elevated serum β-hCG level of more than 10 mIU/ml. Chemical pregnancy rate was defined as the number of chemical pregnancy divided by the number of total transfer cycles for each group.
[d]Clinical pregnancy was defined as a pregnancy documented by ultrasound at 6–8 gestational weeks that shows a gestational sac in the uterus. Clinical pregnancy rate was defined as the number of clinical pregnancy divided by the number of total transfer cycles for each group.
[e]Ongoing pregnancy was defined as a pregnancy documented by ultrasound at 12 gestational weeks that shows the presence of fetal heartbeat. Ongoing pregnancy was defined as the number of ongoing pregnancy divided by the number of total transfer cycles for each group.
[f]Live birth was defined as the delivery of one or more infants with any signs of life after 28 weeks of gestation. Live birth rate (% per transferred cycle) was defined as the number of live birth divided by the number of total transfer cycles for each group.

termination rate due to fetal defect ($p_{trend} = 0.225$). Notably, the fertilization rate decreased as the number of oocytes retrieved increased (Group I: from 83.75% to 70.69%, Group II: from 78.67% to 70.07%, Group III: from 75.62% to 68.93%; Figure S1A). When retrieving less than 10 oocytes, the fertilization rate in Group I was significantly higher than other groups. However, the embryo cleavage rate and the transferrable embryo rate showed no association with the number of oocytes retrieved (Figure S1B–C).

Maternal demographic characteristics and reproductive history of women who delivered live babies were similar among three groups (see Table 3), while distribution of COH protocols and number of oocytes retrieved showed different in both singleton and multiple deliveries ($p < 0.001$; Table S1). When it comes to FET procedures, no difference was found among three groups (Table S2).

https://doi.org/10.1017/S2040174419000679 Published online by Cambridge University Press

Table 3. Maternal characteristics of pregnancies carried to term following transferring embryos with different E2 exposure

	E2 level on the day of hCG trigger (pmol/l)			
	Group I: <10,000 (*N* = 1064)	Group II: 10,000–15,000 (*N* = 690)	Group III: >15,000 (*N* = 1123)	
	No. (%)	No. (%)	No. (%)	*P*
Maternal sociodemographic characteristics				
Age at oocyte retrieval, Mean ± SD, years	30.90 ± 4.11	30.91 ± 3.76	30.95 ± 3.33	0.942
Age at embryo transfer, Mean ± SD, years	30.98 ± 4.08	30.99 ± 3.76	31.04 ± 3.29	0.576
Pregestational BMI, Mean ± SD, kg/m^2	22.26 ± 3.00	21.99 ± 3.15	22.06 ± 3.02	0.259
Residence				
Local residents	631 (59.30)	406 (58.84)	667 (59.39)	0.963
Migrants	433 (40.70)	284 (41.16)	456 (40.61)	
Education attainment				
Primary school or lower	13 (1.22)	11 (1.59)	20 (1.78)	0.347
Middle school	141 (13.25)	104 (15.07)	167 (14.87)	
High school	137 (12.88)	89 (12.90)	156 (13.89)	
Collage or above	773 (72.65)	486 (70.43)	780 (69.46)	
Occupation				
Employed	722 (67.86)	470 (68.12)	761 (67.76)	0.880
Self-employed	231 (21.71)	145 (21.01)	238 (21.19)	
Unemployed	111 (10.43)	75 (10.87)	124 (11.04)	
Smoking during pregnancy				
No	1052 (98.87)	685 (99.28)	1109 (98.75)	0.778
Yes	12 (1.13)	5 (0.72)	14 (1.25)	
History of reproduction				
Parity				
0	963 (90.51)	648 (93.91)	1043 (92.88)	0.072
1	100 (9.40)	42 (6.09)	80 (7.12)	
≥2	1 (0.09)	0 (0.00)	0 (0.00)	
Number of previous abortions				
0	713 (67.01)	452 (65.51)	719 (64.02)	0.315
1–2	323 (30.36)	211 (30.58)	368 (32.77)	
≥3	28 (2.63)	27 (3.91)	36 (3.21)	
Previous ectopic pregnancy				
No	945 (88.82)	603 (87.39)	975 (86.82)	0.157
Yes	119 (11.18)	87 (12.61)	148 (13.18)	
Duration of infertility				
1–2	430 (40.41)	281 (40.72)	466 (41.50)	0.272
3–4	339 (31.86)	218 (31.59)	379 (33.75)	
≥5	295 (27.73)	191 (27.68)	278 (24.76)	
Primary infertility				
No	629 (59.12)	425 (61.59)	659 (58.68)	0.823
Yes	435 (40.88)	265 (38.41)	464 (41.32)	
Causes of infertility				
Tubal infertility	432 (39.76)	279 (40.43)	480 (42.74)	0.339

(*Continued*)

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Table 3. (*Continued*)

	E2 level on the day of hCG trigger (pmol/l)			
	Group I: <10,000 (*N* = 1064)	Group II: 10,000–15,000 (*N* = 690)	Group III: >15,000 (*N* = 1123)	
	No. (%)	No. (%)	No. (%)	*P*
Anovulatory	69 (6.48)	39 (5.65)	89 (7.93)	
Endometriosis	45 (4.23)	17 (2.46)	21 (1.87)	
Male factor infertility	30 (2.82)	17 (2.46)	43 (3.83)	
Unexplained infertility	230 (21.62)	154 (22.32)	200 (17.81)	
Combined[a]	267 (25.09)	184 (26.67)	290 (25.82)	

BMI, body mass index.
[a]Combined was defined as two or more infertile causes mentioned above.

A multivariable analysis of pregnancy complications is shown in Table 4. After adjusting for confounding factors, higher levels of E2 on the day of hCG trigger were found to be associated with an increased risk of preterm birth in singleton deliveries ($aOR_1 = 1.93$, 95% CI: 1.22–3.06; $aOR_2 = 2.05$, 95% CI: 1.33–3.16). Additionally, there were comparable proportions of cases with gestational diabetes mellitus, hypertensive disorder, intrahepatic cholestasis of pregnancy, meconium staining of the amniotic fluid, and caesarean deliveries among the three groups in both singleton and multiple deliveries.

Table 5 reports the associations between neonatal outcomes and E2 level on the day of hCG trigger. As the level of E2 increased on the day of hCG trigger, the risk of LBW births following FET also significantly increased in both singleton and multiple deliveries (singleton: $aOR_1 = 1.27$, 95% CI: 0.75–2.26; $aOR_2 = 1.94$, 95% CI: 1.18–3.20; multiples: $aOR_1 = 1.51$, 95% CI: 1.01–2.26; $aOR_2 = 1.86$, 95% CI: 1.02–3.40). Additionally, increased risks of SGA were found in groups with higher levels of E2 in singletons ($aOR_1 = 2.01$, 95% CI: 1.30–3.11; $aOR_2 = 2.51$, 95% CI: 1.69–3.74). A similar effect was also observed in multiple deliveries ($aOR_1 = 1.58$, 95% CI: 1.03–2.45; aOR2 = 1.99, 95% CI: 1.05–3.84). Due to the impact of COH on maternal E2 levels, a subgroup analysis based on COH protocol was also carried out (Table S3). Women experienced either GnRH-agonist regimen or GnRH-antagonist regimen showed higher risks of SGA as E2 level on the day of hCG trigger increased in singletons (GnRH-agonist regimen: $aOR_2 = 2.16$, 95% CI: 1.13–4.11; GnRH-antagonist regimen: $aOR_2 = 2.40$, 95% CI: 1.40–4.11). There was no evidence of differences among the study groups in terms of macrosomia in singletons, and no cases of macrosomia was found in the three groups among multiples. No association was observed between LGA and E2 level on the day of hCG trigger.

Discussion

In this retrospective cohort of 10,581 FET cycles, we found that high E2 levels on the day of hCG trigger was associated with lower percentage of clinical pregnancy, ongoing pregnancy, and live birth, as well as an increased risk of early pregnancy loss after FET. In addition, newborns after FET with higher E2 levels during COH showed an increased risk of preterm birth in singletons, along with an increased risk of LBW and SGA in both singletons and multiples. These findings strongly suggest that COH-induced high E2 may have adverse effects on oocyte development and maturation, and subsequently increase the risk of SGA following FET.

The most critical discrepancy between fresh embryo transfer and FET is the COH-induced persistent changes in the hormonal milieu after embryo transfer, especially supraphysiological E2 levels.[18] FET is believed to enable patients who have experienced COH to recover to their initial hormone levels in the next or further cycles, thus providing a relatively normal hormonal milieu during pregnancy.[8] However, in cases of women who were transferred fresh embryo in the current cycle soon after COH, incidence of SGA was reported to be higher than FET.[12,19,20] Moreover, large cohort studies confirmed that the alternation of hormone milieu by COH is one of the interpretations of risk of LBW or SGA in fresh embryo transfer.[4,21,22] Notably, a similar association between COH and LBW or SGA was also found in FET population in this study, regardless of the fact that FET cycles provide a significantly lower level in E2 before embryo transfer than that during COH (E2: 2578 [1518–4171] pmol/l vs. 12796 [7641–17800] pmol/l, $p < 0.001$, data not shown in tables). Thus, supraphysiological E2 level induced by COH is believed to have influence not only on embryos during early pregnancy, but also on the oocytes during COH. Furthermore, it is worth noting that the rate of fertilization was negatively correlated with the number of oocyte retrieval. A higher number of oocytes retrieved is regarded as an indication of excessive E2 levels during COH,[23,24] as estrogen stimulates follicle development and is synthetized and secreted locally to a higher level by granulosa cells.[10] According to our findings, in spite of the fact that a certain level of estrogen is essential for the growth of follicles, milder ovarian stimulation regimens with less than 10 oocytes retrieved should be advocated to avoid the excessive response of ovary and impairment on oocyte maturation following IVF.

The process of oocyte maturation is complicated, which involves phenotypic changes, reorganization of cytoplasmic structures, organelle formation, and changes in specific molecules related to fertilization and embryo development. Evidence from animal models and human has indicated that certain transient environmental influences could produce persistent changes in epigenetic marks, thus regulating fetal growth and development in the future life.[25–27] Early in the 2001, Van der Auwera *et al.* transferred blastocysts from stimulated female mice and naturally cycling controls to non-stimulated foster mothers separately and found that superovulation caused delayed embryonic development and a pronounced fetal growth restriction.[28] Furthermore, experiments from nonhuman primates suggest an activated E2 signaling

https://doi.org/10.1017/S2040174419000679 Published online by Cambridge University Press

Table 4. Pregnancy complications of pregnancies carried to term following transferring embryos with different E2 exposure

	Singleton delivery					Multiple deliveries				
	E2 level on the day of hCG trigger (pmol/l)					E2 level on the day of hCG trigger (pmol/l)				
	Group I: <10,000	Group II: 10,000–15,000	Group III: >15,000	aOR_1 (95% CI)[a]	aOR_2 (95% CI)[a]	Group I: <10,000	Group II: 10,000–15,000	Group III: >15,000	aOR_1 (95% CI)[a]	aOR_2 (95% CI)[a]
	(*N* = 886)	(*N* = 523)	(*N* = 821)			(*N* = 178)	(*N* = 167)	(*N* = 302)		
	No. (%)	No. (%)	No. (%)	Group II vs. I	Group III vs. I	No. (%)	No. (%)	No. (%)	Group II vs. I	Group III vs. I
Gestational diabetes mellitus										
No	808 (91.20)	473 (90.44)	746 (90.86)	Reference	Reference	157 (88.20)	144 (86.23)	264 (87.42)	Reference	Reference
Yes	78 (8.80)	50 (9.56)	75 (9.14)	1.05 (0.71–1.55)	0.96 (0.67–1.38)	21 (11.80)	23 (13.77)	38 (12.56)	1.06 (0.54–2.09)	0.84 (0.42–1.66)
Hypertensive disorder										
No	751 (84.76)	441 (84.32)	693 (84.41)	Reference	Reference	141 (79.21)	131 (78.44)	238 (78.81)	Reference	Reference
Gestational hypertension	52 (5.87)	39 (7.46)	58 (7.06)	1.35 (0.86–2.10)	1.37 (0.90–2.10)	11 (6.18)	11 (6.59)	22 (7.28)	0.92 (0.36–2.33)	0.74 (0.28–1.92)
Mild preeclampsia	54 (6.09)	26 (4.97)	41 (4.99)	0.85 (0.51–1.39)	0.82 (0.52–1.29)	13 (7.30)	12 (7.19)	21 (6.95)	0.72 (0.30–1.72)	0.62 (0.26–1.49)
Wild preeclampsia	29 (3.27)	17 (3.25)	29 (3.53)	0.91 (0.48–1.70)	0.87 (0.50–1.53)	13 (7.30)	13 (7.78)	21 (6.95)	1.19 (0.50–2.84)	1.07 (0.44–2.59)
Intrahepatic cholestasis of pregnancy										
No	855 (96.50)	502 (95.98)	785 (95.62)	Reference	Reference	168 (94.38)	157 (94.01)	287 (95.03)	Reference	Reference
Yes	31 (3.50)	21 (4.02)	36 (4.38)	1.13 (0.63–2.02)	1.26 (0.74–2.13)	10 (5.62)	10 (5.99)	15 (4.97)	1.03 (0.39–2.673)	1.06 (0.40–2.78)
Meconium staining of the amniotic fluid										
No	739 (83.41)	428 (81.84)	689 (83.92)	Reference	Reference	159 (89.33)	150 (89.82)	269 (89.07)	Reference	Reference
Yes	147 (16.59)	95 (18.16)	132 (16.08)	1.06 (0.79–1.42)	0.91 (0.69–1.21)	19 (10.67)	17 (10.18)	33 (10.93)	0.81 (0.39–1.70)	0.67 (0.32–1.42)
Preterm birth[b]										
No	839 (94.70)	476 (91.01)	740 (90.13)	Reference	Reference	85 (47.75)	85 (50.90)	141 (46.69)	Reference	Reference
Preterm	40 (4.51)	42 (8.03)	69 (8.40)	1.93 (1.22–3.06)	2.05 (1.33–3.16)	88 (49.44)	76 (45.51)	147 (48.68)	0.78 (0.49–1.24)	0.88 (0.55–1.39)
Very preterm	7 (0.79)	5 (0.96)	12 (1.46)	1.17 (0.36–3.80)	1.56 (0.57–4.33)	5 (2.81)	6 (3.59)	14 (4.64)	1.32 (0.35–4.94)	1.71 (0.49–5.91)
Mode of delivery										
Vaginal	336 (37.92)	211 (40.34)	290 (35.32)	Reference	Reference	8 (4.49)	3 (1.80)	9 (2.98)	Reference	Reference
Cesarean section	550 (62.08)	312 (59.66)	531 (64.88)	0.86 (0.68–1.08)	0.96 (0.78–1.19)	170 (95.51)	164 (98.20)	293 (97.02)	2.88 (0.71–11.69)	1.65 (0.49–5.55)
Vanishing twin										
No	842 (93.00)	484 (92.54)	751 (91.47)	Reference	Reference					
Yes	62 (7.00)	39 (7.46)	70 (8.53)	1.01 (0.66–1.55)	1.22 (0.83–1.79)					

aOR, adjusted odds ratio; CI, confidence interval.
[a]aOR was adjusted for age at oocyte retrieval, age at embryo transfer, pregestational BMI, COH protocol, number of oocytes retrieved.
[b]Preterm was defined as delivery of baby before 37 gestational weeks of pregnancy, and very preterm was defined as delivery of baby between 28 and 32 gestational weeks of pregnancy.

https://doi.org/10.1017/S2040174419000679 Published online by Cambridge University Press

Table 5. Outcomes of neonates born following transferring embryos with different E2 exposure

	Singleton delivery					Multiple deliveries				
	E2 level on the day of hCG trigger (pmol/l)					E2 level on the day of hCG trigger (pmol/l)				
	Group I: <10,000	Group II: 10,000–15,000	Group III: >15,000			Group I: <10,000	Group II: 10,000–15,000	Group III: >15,000		
	(*N* = 886)	(*N* = 523)	(*N* = 821)	aOR_1 (95% CI)[a]	aOR_2 (95% CI)[a]	(*N* = 357)	(*N* = 334)	(*N* = 604)	aOR_1 (95% CI)[a]	aOR_2 (95% CI)[a]
	No. (%)	No. (%)	No. (%)	Group II vs. I	Group III vs. I	No. (%)	No. (%)	No. (%)	Group II vs. I	Group III vs. I
Gestational age (Mean ± SD, weeks)	38.31 ± 1.60	38.05 ± 1.74	38.05 ± 1.89			35.92 ± 1.55	35.93 ± 1.69	35.79 ± 1.99		
Gender										
Male	446 (50.34)	258 (49.33)	434 (52.86)	Reference	Reference	182 (50.98)	174 (52.10)	317 (52.48)	Reference	Reference
Female	440 (49.66)	265 (50.67)	387 (47.14)	1.06 (0.84–1.32)	0.91 (0.74–1.12)	175 (49.02)	160 (47.90)	287 (47.52)	1.03 (0.75–1.41)	1.04 (0.65–1.67)
Birthweight										
<2500 g	39 (4.40)	34 (6.50)	79 (9.62)	1.27 (0.71–2.26)	1.94 (1.18–3.20)	159 (44.54)	162 (48.50)	318 (52.65)	1.51 (1.01–2.26)	1.86 (1.02–3.40)
2500–4000 g	778 (87.81)	455 (87.00)	684 (83.31)	Reference	Reference	198 (55.46)	172 (51.50)	286 (47.35)	Reference	Reference
>4000 g	69 (7.79)	34 (6.50)	58 (7.06)	0.92 (0.59–1.42)	1.07 (0.72–1.59)	0 (0.00)	0 (0.00)	0 (0.00)	NA	NA
Birthweight for gestational age										
SGA	44 (4.97)	50 (9.56)	106 (12.91)	2.01 (1.30–3.11)	2.51 (1.69–3.74)	60 (16.81)	65 (19.46)	135 (22.35)	1.58 (1.03–2.45)	1.99 (1.05–3.84)
AGA	689 (77.77)	380 (72.66)	583 (71.01)	Reference	Reference	292 (81.79)	265 (79.34)	458 (75.83)	Reference	Reference
LGA	153 (17.27)	93 (17.78)	132 (16.08)	1.11 (0.83–1.50)	0.98 (0.74–1.30)	5 (1.40)	4 (1.20)	11 (1.82)	0.88 (0.20–3.85)	0.83 (0.09–7.57)

aOR, adjusted odds ratio; CI, confidence interval; NA, not accessible; AGA, appropriate for gestational age; SGA, small for gestational age.
[a]aOR was adjusted for age at embryo transfer, pregestational BMI, COH protocol, number of oocytes retrieved, number of embryo.

involved in the regulation of trophoblast differentiation in early embryo development,[29,30] subsequently leading to insufficient invasion of trophoblast and uteroplacental vessel remodeling. As a result, the blood flow is impaired to the placenta, which might cause several adverse obstetric outcomes, for example, miscarriage, preterm birth, preeclampsia, and SGA.[31] Although no evidence of an effect was found in regard to gestational hypertensive disorders, an increased risk of preterm birth and SGA after deliveries was confirmed in this study.

Recent years, a growing number of studies indicated that the cause of many chronic diseases in adulthood including obesity, diabetes, hypertension, and cardiovascular diseases can be traced back to gametic and embryonic development stage, and exploring origin of the disease contributes to early intervention and treatment.[32–34] However, due to the ethical concerns, interventional study is not permitted on human gametes and embryos. In this study, FET provides us an ideal model to explore the association between the follicle microenvironment and oocyte development as well as further pregnancy outcomes. As this is a hospital-based retrospective cohort study, several confounders related to the medical procedures have been taken into consideration. It is, therefore, not possible to rule out unknown confounders which might influence the risk of obstetric complications and newborn birthweight, such as nutrient intake and physical activities during pregnancy. Despite the limitations, our study is the first epidemiological investigation with regard to the impact of oocyte exposure to transient increase in E2 during COH on pregnancy outcomes, as well as the subsequent perinatal outcomes, without the interference of abnormal maternal hormone milieu during implantation and early pregnancy. The large sample size provided sufficient power to detect small but clinically significant effects. Evidence provided by this study regarding the potential adverse effects of supraphysiological E2 should be taken into consideration when formulating treatment options.

In summary, an increased level of E2 during COH is associated with a decreased pregnancy rate, as well as an increased frequency of early miscarriage following FET. Furthermore, pregnancies conceived from frozen-thawed embryos with supraphysiological E2 exposure during COH may also at increased risk of preterm birth, LBW, and SGA. Therefore, milder COH protocols should be used to avoid the exposure of a supraphysiological level of E2 to the gametes and embryos.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S2040174419000679

Acknowledgements. We thank Nigel Rollins, Maternal, Newborn, Child and Adolescent Health, World Health Organization, for contributions of critical revision of the manuscript. We also thank Prof. Shu-Min Duan, Zhejiang University School of Medicine, for the contributions of study designing.

Financial support. This study was supported by the National Key Research and Development Program of China (2018YFC1002804, 2016YFC1000203), the National Natural Science Foundation of China (81671412, 81661128010), Foundation of Shanghai Municipal Commission of Health and Family Planning (20144Y0110), and Clinical Skills Improvement Foundation of Shanghai Jiaotong University School of Medicine (JQ201717).

Conflict of interest. None.

Ethical standards. Ethical approval for the study was obtained from the Institutional Review Board of IPMCHH (GKLW-2016-21). Written informed consent was obtained from all participants before inclusion.

Author Contributions. He-Feng Huang conceived the hypothesis. Yan-Ting Wu designed the study. Chen-Chi Duan and Cheng Li drafted the manuscript. Yi-Chen He, Jing-Jing Xu, and Chao-Yi Shi conducted the statistical analysis and participated in the discussion. Hong-Tao Hu, Yun-Fei Su, and Lei Chen participated in data collection. Ya-Jing Tan conducted embryo manipulation. Zhi-Wei Liu, Jian-Zhong Sheng, William Fraser, and He-Feng Huang critically revised the manuscript for important intellectual content. All authors commented on the drafts and approved the final draft.

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