

# Temporary Increased LDL-C in Offspring with Extreme Elevation of Maternal Preconception Estradiol: A Retrospective Cohort Study

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**Objective:** To investigate the effect of maternal estradiol (E<sub>2</sub>) elevation on long-term metabolic manifestations in the offspring.

**Study Design and Setting:** This was a retrospective cohort study. Overall, 3690 children conceived by in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) between July 2014 and December 2017 were recruited and divided into four groups categorized by maternal E<sub>2</sub> quartiles (Q1, <2420; Q2, 2420–3839; Q3, 3839–5599; and Q4, ≥5599 pg/mL). The metabolic profiles were measured during childhood. Linear mixed models were used to evaluate the association between maternal E<sub>2</sub> elevation and metabolic phenotypes of the offspring.

**Results:** Lipoprotein cholesterol (LDL-C) was significantly higher in the highest quartile group than in the lowest quartile group during infancy (adjusted mean difference [95% confidence interval, CI]: 0.11 [0.02, 0.20], P = 0.005), but the difference disappeared in the later childhood phase. In children born after fresh embryo transfer, LDL-C showed an increasing trend with the increase in maternal E<sub>2</sub> level (adjusted mean difference [95% CI]: Q2 vs Q1, -0.01 [-0.11, 0.08], Q3 vs Q1, 0.06 [-0.04, 0.15], Q4 vs Q1, 0.10 [0, 0.20]). Other metabolic variables were comparable across increasing quartiles of maternal E<sub>2</sub> levels.

**Conclusion:** This study demonstrates a temporary increase in LDL-C levels in infants with higher levels of maternal preconception E<sub>2</sub> levels. However, the long-term safety of hyperestrogens after ovarian stimulation in the next generation is favorable. The mechanism underlying the transiently increased metabolic dysfunction risk in infants conceived by IVF/ICSI requires investigation in future studies.

**Keywords:** offspring, supraphysiologic estradiol, controlled ovarian hyperstimulation, assisted reproductive technology

## Introduction

Previous studies, including ours, indicated that offspring conceived through in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) showed less favorable metabolic health manifesting as differential peripheral body fat deposit,<sup>1,2</sup> elevated blood pressure,<sup>3</sup> deteriorate gluco-metabolism,<sup>4-6</sup> raised triglycerides (TG),<sup>7</sup> and decreased low-density lipoprotein cholesterol (LDL-C) levels.<sup>5</sup> According to the DOHaD theory, metabolic changes in later life can be traced back to abnormal exposure during early life.<sup>8,9</sup> Therefore, IVF/ICSI procedures were considered related to exposure in this population. Since infertile couples would not have babies without IVF/ICSI treatment, determining the key factors that conferred metabolic risk in the next generation was essential.

Controlled ovarian hyperstimulation (COH) is the key procedure in IVF/ICSI treatment, and the fundamental differential exposure between IVF/ICSI offspring and those nature conceived.<sup>10</sup> Compared with the natural cycle, maternal serum estradiol (E<sub>2</sub>) levels increased more than tenfold in the COH cycle.<sup>11</sup> It has been reported that higher maternal E<sub>2</sub> levels on human chorionic gonadotropin (hCG) trigger days are associated with the risk of low birth weight (LBW) in the offspring not only after fresh<sup>12</sup> but also after frozen embryo transfer (ET).<sup>13</sup> LBW is well accepted as a risk factor for poor metabolic outcomes later in life.<sup>14</sup> Moreover, elevated maternal serum E<sub>2</sub> levels throughout pregnancy are directly associated with insulin resistance and dyslipidemia in newborns.<sup>15,16</sup> However, animal studies reported that mice conceived by COH alone did not exhibit any metabolic dysfunction in childhood<sup>6</sup> and even showed reduced lipid accumulation in the peripheral metabolic organs of old mice.<sup>16</sup> Unfortunately, human data on the long-term metabolic profiles of offspring conceived through IVF/ICSI are limited. Only one study reported that prenatal high E<sub>2</sub> exposure increased the fasting insulin and homeostatic model assessment for insulin resistance (HOMA-IR) index of children conceived by IVF/ICSI.<sup>15</sup>

This retrospective cohort study aimed to investigate the association between maternal E<sub>2</sub> levels and the long-term metabolic profiles of children conceived through IVF/ICSI at ages 2–9. The results would help to target the risk factors of metabolic disturbance in IVF/ICSI offspring and provide valuable evidence on the intergenerational safety of ovarian stimulation in counseling couples with infertility before IVF/ICSI treatment.

## Materials and Methods

### Study Population

This retrospective cohort study was performed at the Center for Reproductive Medicine, Cheeloo College of Medicine, Shandong University. A total of 3690 singleton children who conceived by IVF/ICSI treatment between July 2014 and December 2017 were recruited. Children born after oocyte or embryo donation were excluded from this study. All participants were followed up at least once in their childhood (aged 2 to 9 years), which led to a total of 5238 visits. The study was approved by the Reproductive Medicine Ethics Committee of the Hospital for Reproductive Medicine Affiliated to Shandong University (Ethics approval number: NO. 2014).<sup>17</sup> The parents signed informed consent forms with the assent of the child.

### Outcome Assessment

The enrolled children were recalled at least once during their childhood phase. All the outcome assessments were performed at each visit. The height ( $\pm 0.1$  cm) and weight ( $\pm 0.1$  kg) of the offspring were measured twice using a stadiometer and electronic scale with a light cloth and no shoes. The BMI was calculated as weight (kg)/[height (m)]<sup>2</sup>. Blood samples of the offspring were collected after overnight fasting and stored at  $-80^{\circ}\text{C}$  until the tests. Fasting blood glucose (FBG) levels were analyzed using the hexokinase method (Cobas c702 instrument; Roche Diagnostics, Germany). Fasting insulin was measured in the serum using an electrochemiluminescence immunoassay (Cobas e601 instrument; Roche Diagnostics, Germany). Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and LDL-C levels were measured in serum using a homogeneous assay (Cobas c702 instrument; Roche Diagnostics, Germany). HOMA-IR was calculated as [insulin (mIU/L) FBG (mmol/L)]/22.5. HOMA of beta-cell function (HOMA- $\beta$ ) was calculated using the formula  $[20 \times \text{insulin (mIU/mL)}] / [\text{FBG (mmol/L)} - 3.5]$ .

### Exposure Assessment

The IVF/ICSI protocols have been described in detail in our previous study.<sup>17</sup> Maternal E<sub>2</sub> levels were tested using chemiluminescence immunoassays (Roche Diagnostics, Germany) on hCG trigger days, with intra- and inter-assay coefficients of variation of  $< 10\%$ . All mother-offspring pairs were grouped into four quartiles: Q1 (E<sub>2</sub>  $< 2420$  pg/mL), Q2 ( $2420 \leq E_2 < 3839$  pg/mL), Q3 ( $3839 \leq E_2 < 5599$  pg/mL), and Q4 (E<sub>2</sub>  $\geq 5599$  pg/mL).

### Covariate Assessment

Pre-pregnancy height ( $\pm 0.1$  cm) and weight ( $\pm 0.1$  kg) of parents were measured at enrolment using a stadiometer and electronic scale to calculate BMI. Information on the parental date of birth, educational level, and parity (0 or 1) was

obtained using a standardized questionnaire during a face-to-face interview. Parental age at delivery was calculated based on the date of parental birth and delivery. The IVF/ICSI procedures were extracted by reviewing the medical records. In addition to ovarian stimulation, IVF/ICSI procedures included ICSI (no or yes), cycle type (fresh or frozen), number of embryos transfer (one or more), stage of ET (cleavage or blastocyst), preimplantation genetic testing (PGT) (no or yes), and assisted hatching (no or yes). Perinatal information was recorded based on the birth certificate, including date of delivery, sex (male or female), birth weight, gestational age, and caesarean birth (no or yes). The dietary intake of the children was obtained using a standardized questionnaire completed by their parents. Energy intake was calculated based on the dietary intake when the offspring were above 4 years of age.

## Statistical Analysis

All data were analyzed using R3.6.3. Categorical variables were presented as counts (proportions). For continuous variables, normality plots and the Shapiro–Wilk test were used for normality testing. Continuous variables are presented as mean  $\pm$  standard deviation when normally distributed and as median (25th–75th percentile) when not normally distributed. Baseline characteristics among the four groups were compared using the Kruskal–Wallis test for non-normally distributed continuous variables and Fisher’s exact test for categorical variables. Linear mixed effects regression was used to determine the estimated mean difference (MD) and 95% confidence intervals (CIs) of outcomes for each quartile of  $E_2$  levels, with the lowest quartile (Q1) always used as a reference. A linear mixed-effects regression model was used to adjust for the confounding effect of repeated measurements in the same child at different time points.<sup>18</sup> Model 1 tested the crude unadjusted values. Model 2 was used to control for potential confounding factors including maternal age, BMI, paternal age, BMI, cycle type, number of ET, stage of ET, offspring gestational age, birth weight, sex, and age at the visit. Since the distribution of insulin, HOMA-IR, and HOMA- $\beta$  was skewed,  $\log_e$  transformation was performed prior to the application of regression models. A P-value  $<0.017$  was regarded as statistically significant (Bonferroni adjustment, 0.05/3). To test the robustness of the results, we further stratified the mother-offspring pairs according to cycle type (fresh and frozen ET) and offspring age (2–3, 4–5, and 6–9 years).

## Results

We finally included 3690 mother-offspring pairs and 5238 flow-up visits in this study [maternal age, 30.8 (28.2, 34.0) years; maternal BMI, 22.7 (20.7, 25.1)  $\text{kg}/\text{m}^2$ ; paternal age, 31.6 (28.6, 34.9) years; paternal BMI 25.4 (22.8, 28.0)  $\text{kg}/\text{m}^2$ ; male offspring, 1954 (53.0%); offspring age, 4.1 (3.1, 5.6)  $\text{kg}/\text{m}^2$ ]. The baseline characteristics of the patients are presented in [Table 1](#). Parental age and maternal BMI were the highest in the Q1 group. There were no significant differences in the parental education level or parity between the groups. The median maternal  $E_2$  levels were 1770, 3111, 4329, and 7043  $\text{pg}/\text{mL}$  in the four groups. The lowest median gonadotropin dose was observed in the top quartile (Q4) group, with the highest ovarian stimulation duration. Transfer of frozen embryos, single embryos, and blastocysts was most prevalent in the Q4 group. The proportions of ICSI, PGT, and assisted hatching were similar among the four groups. The gestational age of the Q1 group was the lowest. There were no significant differences among the groups in terms of birth weight, caesarean birth, and sex. During the follow-up visits, the highest median offspring age was 4.5 years in the Q4 group. The dietary intake of the children among the groups was comparable.

[Table 2](#) shows the metabolic variables of children grouped by maternal  $E_2$  levels. No differences were found in BMI, FBG, insulin, HOMA-IR, HOMA- $\beta$ , TC, TG, LDL-C, or HDL-C across increasing quartiles of maternal  $E_2$  levels. The results were maintained after adjusting for confounding factors including parental age, BMI, cycle type, number, stage of ET, offspring gestational age, birth weight, sex, and age at the visit. The findings were consistent with the main results of subgroup analysis stratified by cycle type (fresh and frozen ET) ([Figure 1](#)). However, a trend of gradient increase in LDL-C along with maternal  $E_2$  was observed in children born after fresh ET [adjusted mean difference (95% CI): Q2 vs Q1,  $-0.01$  ( $-0.11, 0.08$ ); Q3 vs Q1,  $0.06$  ( $-0.04, 0.15$ ), Q4 vs Q1,  $0.10$  ( $0, 0.20$ )]. We further stratified the offspring into three subgroups according to age (2–3, 4–5, and 6–9 years). The infant characteristics are listed in [Table 3](#). Compared to the Q1 group, LDL-C was significantly increased in the Q4 group after adjusting for confounding factors during infancy [adjusted mean difference (95% CI):  $0.11$  ( $0.02, 0.20$ ),  $P=0.005$ ; [Table 4](#)]. However, the parameters during childhood were comparable among the groups [Tables S1–2](#).

**Table 1** Baseline Characteristics of Study Population

	Quartiles Based on Maternal E <sub>2</sub>				p value	Total (n=3690)
	Q1 (n=923) E <sub>2</sub> <2420	Q2 (n=922) 2420≤E <sub>2</sub> <3839	Q3 (n=922) 3839≤E <sub>2</sub> <5599	Q4 (n=923) E <sub>2</sub> ≥5599		
<b>Parental characteristics</b>						
Maternal age at delivery, years	32.1(28.7, 34.9)	30.8(28.3, 33.9)	30.6(28.1, 33.7)	30.3(27.7, 33.1)	<b>&lt;0.001</b>	30.8(28.2, 34.0)
Maternal BMI, kg/m <sup>2</sup>	23.4(21.3, 26)	22.8(20.8, 25.5)	22.3(20.4, 24.6)	22.1(20.3, 24.3)	<b>&lt;0.001</b>	22.7(20.7, 25.1)
Maternal education level (college or higher), n (%)	275(29.8)	276(29.9)	295(32.0)	275(29.8)	0.440	1121(30.4)
Nulliparity, n (%)	778(84.3)	805(87.3)	810(87.9)	812(88.0)	0.063	3205(86.9)
Paternal age at delivery, years	32.4(29.1, 35.9)	31.7(28.4, 35.2)	31.5(28.6, 34.6)	30.8(28.1, 34.1)	<b>&lt;0.001</b>	31.6(28.6, 34.9)
Paternal BMI, kg/m <sup>2</sup>	25.3(22.6, 27.9)	25.6(23.1, 28.1)	25.4(22.7, 28.1)	25.4(22.8, 27.8)	0.453	25.4(22.8, 28.0)
Paternal education level (college or higher), n (%)	305(33.0)	334(36.2)	354(38.4)	316(34.2)	0.172	1309(35.5)
<b>IVF/ICSI Characteristics<sup>†</sup></b>						
E <sub>2</sub> levels on hCG trigger day, pg/mL	1770(1399, 2094)	3111(2743, 3493)	4329(4300, 4991)	7043(6260, 8231)	<b>&lt;0.001</b>	3839(2416, 5599)
Days of ovarian stimulation, days <sup>†</sup>	10(8, 11)	10(9, 11)	10(9, 11)	10(9, 11)	<b>&lt;0.001</b>	10(9, 11)
Gonadotropin dose, IU <sup>†</sup>	1850(1350, 2475)	1800(1350, 2400)	1775(1350, 2275)	1575(1313, 2050)	<b>&lt;0.001</b>	1725(1350, 2250)
Cycle type (Frozen), n (%)	249(27.0)	354(38.4)	472(51.2)	573(62.1)	<b>&lt;0.001</b>	1648(44.7)
Number of embryos transfer>1, n (%)	557(60.3)	613(66.5)	571(61.9)	536(58.1)	<b>0.002</b>	2277(61.7)
Stage of embryo transfer (Blastocyst), n (%)	310(33.6)	356(38.6)	469(50.9)	563(61.0)	<b>&lt;0.001</b>	1698(46.0)
ICSI used, n (%)	274(29.8)	281(30.5)	308(33.4)	304(32.9)	0.263	1167(31.7)
PGT used, n (%)	28(3.0)	23(2.5)	29(3.1)	25(2.7)	0.828	105(2.8)
Assisted hatching, n (%)	918(99.5)	916(99.3)	915(99.2)	917(99.3)	0.953	3666(99.3)
<b>Perinatal characteristics</b>						
Birthweight, kg	3.5(3.2, 3.8)	3.5(3.2, 3.8)	3.5(3.2, 3.8)	3.5(3.2, 3.8)	0.834	3.5(3.2, 3.8)
Gestational age at birth, weeks	39.1(38.3, 40.0)	39.3(38.6, 40.0)	39.1(38.4, 40.0)	39.3(38.4, 40.0)	<b>0.020</b>	39.1(38.4, 40.0)
Caesarean birth, n (%)	726(78.7)	699(75.8)	681(73.9)	689(74.6)	0.084	2795(75.7)
Sex (male), n (%)	489(53.0)	459(49.8)	515(55.9)	491(53.2)	0.076	1954(53.0)
<b>Children's characteristics and lifestyle factors</b>						
No. of visits	1295	1273	1338	1332	–	5238
Age, years	4.0(3.0, 5.5)	4.0(3.1, 5.5)	4.1(3.0, 5.7)	4.5(3.2, 5.9)	<b>&lt;0.001</b>	4.1(3.1, 5.6)
Intake of food and drink						
2≤age<4						
Vegetables daily, n (%)	515(80.0)	515(83.2)	537(84.3)	484(83.0)	0.203	2051(82.6)
Fruit daily, n (%)	561(87.1)	548(88.5)	581(91.2)	521(89.4)	0.124	2211(89.0)
Dairy food daily, n (%)	273(42.4)	257(41.5)	278(43.6)	236(40.5)	0.716	1044(42.0)
Marine products once or more weekly, n (%)	399(62.0)	371(59.9)	384(60.3)	338(58.0)	0.565	1492(60.1)
4≤age<10						
Energy, kcal/day	1826(1511, 2291)	1797(1462, 2237)	1844(1503, 2292)	1802(1503, 2238)	0.261	1816(1491, 2264)

**Notes:** Data are presented as median (interquartile range) or n (%). Statistical differences were tested using the Kruskal–Wallis test for continuous variables and the Fisher's exact test for categorical variables. Statistical significance was set at P <0.05, and the results are highlighted in bold. <sup>†</sup>For the Q2 group, two data points were missing in the days of ovarian stimulation and gonadotropin dose.

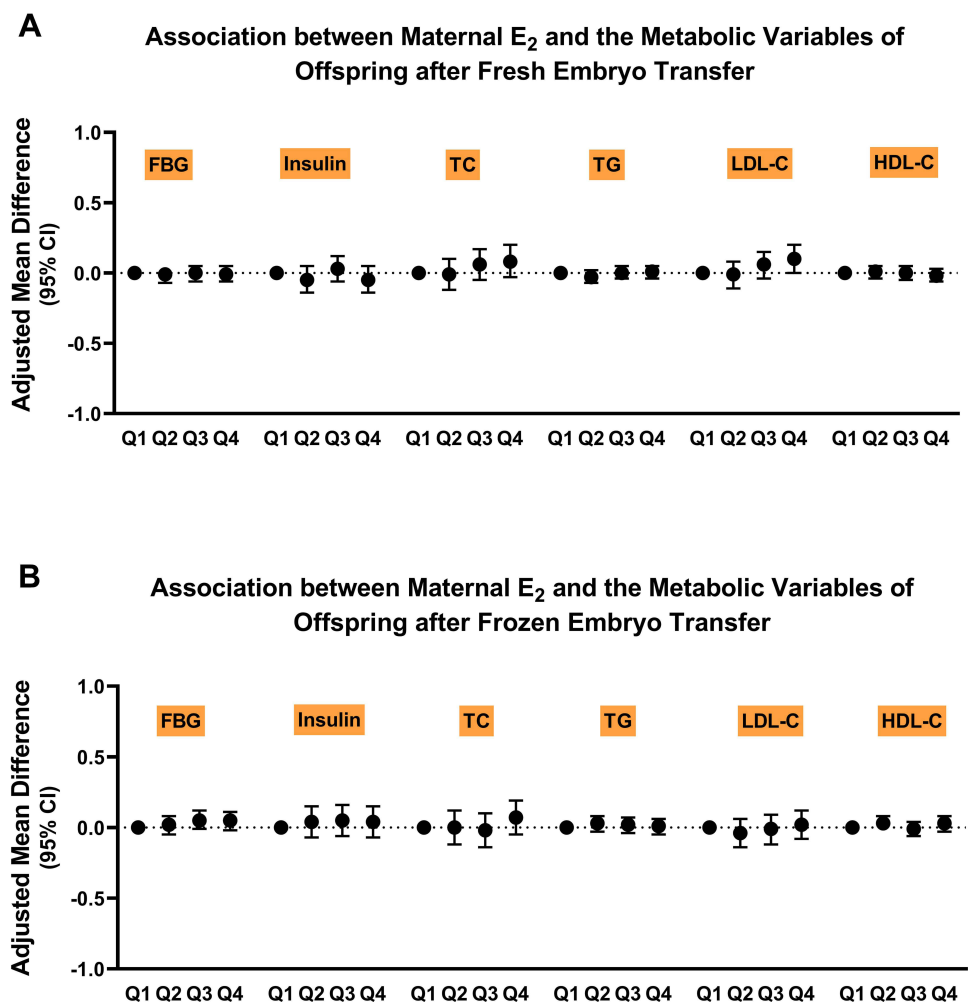
**Abbreviations:** BMI, body mass index; E<sub>2</sub>, estradiol; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; PGT, preimplantation genetic testing.

**Table 2** Mean Differences (95% CI) for Metabolic Variables of Children Across Quartiles of Maternal E<sub>2</sub> Levels

		Quartiles Based on Maternal E <sub>2</sub>						
		Q1 (1295 Visits) E <sub>2</sub> <2420	Q2 (1273 Visits) 2420≤E <sub>2</sub> <3839	p value	Q3 (1338 Visits) 3839≤E <sub>2</sub> <5599	p value	Q4 (1332 Visits) E <sub>2</sub> ≥5599	p value
BMI, kg/m <sup>2</sup>	Crude model	Ref	0.01(−0.25, 0.26)	1.000	0.08(−0.18, 0.33)	0.866	0.08(−0.17, 0.34)	0.830
	Adjusted model	Ref	0.02(−0.22, 0.25)	0.998	0.15(−0.09, 0.39)	0.390	0.10(−0.15, 0.34)	0.750
FBG, mmol/l	Crude model	Ref	−0.01(−0.05, 0.04)	0.978	0(−0.04, 0.05)	0.999	0.03(−0.02, 0.07)	0.339
	Adjusted model	Ref	0(−0.05, 0.04)	0.995	0(−0.04, 0.05)	0.991	0.03(−0.02, 0.07)	0.330
Insulin, pmol/l <sup>†</sup>	Crude model	Ref	0.05(−0.03, 0.13)	0.381	0(−0.07, 0.08)	0.999	0.05(−0.03, 0.12)	0.437
	Adjusted model	Ref	0.04(−0.03, 0.11)	0.509	0.01(−0.06, 0.08)	0.993	0.02(−0.05, 0.09)	0.883
HOMA-IR <sup>†</sup>	Crude model	Ref	0.05(−0.04, 0.13)	0.465	0(−0.08, 0.09)	0.999	0.05(−0.03, 0.13)	0.387
	Adjusted model	Ref	0.04(−0.04, 0.11)	0.582	0.01(−0.07, 0.08)	0.992	0.03(−0.05, 0.10)	0.815
HOMA-β <sup>†</sup>	Crude model	Ref	0.05(−0.01, 0.12)	0.166	0.02(−0.05, 0.09)	0.864	0.02(−0.04, 0.09)	0.781
	Adjusted model	Ref	0.04(−0.02, 0.10)	0.313	0.02(−0.04, 0.09)	0.821	0(−0.07, 0.07)	1.000
TC, mmol/l	Crude model	Ref	−0.02(−0.09, 0.06)	0.962	0.01(−0.07, 0.09)	0.970	0.03(−0.05, 0.11)	0.723
	Adjusted model	ref	0(−0.09, 0.08)	0.999	0.04(−0.04, 0.12)	0.600	0.07(−0.01, 0.16)	0.114
TG, mmol/l	Crude model	Ref	0.01(−0.02, 0.05)	0.790	0.01(−0.02, 0.05)	0.775	0.01(−0.03, 0.04)	0.954
	Adjusted model	Ref	0.01(−0.03, 0.04)	0.907	0.02(−0.02, 0.05)	0.671	0.01(−0.03, 0.05)	0.898
LDL-C, mmol/l	Crude model	Ref	−0.01(−0.08, 0.06)	0.982	0.01(−0.05, 0.08)	0.964	0.03(−0.04, 0.09)	0.769
	Adjusted model	Ref	0(−0.07, 0.07)	0.999	0.04(−0.03, 0.11)	0.487	0.07(0, 0.14)	0.056
HDL-C, mmol/l	Crude model	Ref	−0.01(−0.05, 0.02)	0.839	0(−0.03, 0.03)	1.000	0(−0.03, 0.04)	0.999
	Adjusted model	Ref	0(−0.04, 0.03)	0.984	0(−0.04, 0.03)	0.999	−0.01(−0.04, 0.03)	0.962

**Notes:** Mean differences were obtained using a linear mixed-effects regression model. <sup>†</sup>Loge-transformed Insulin, HOMA-IR, HOMA-β values were considered continuous variables. Adjusted model: adjusted for maternal age, BMI, paternal age, BMI, cycle type, number of embryos transfer, stage of embryo transfer, offspring gestational age, birth weight, sex and age at visit.

**Abbreviations:** BMI, body mass index; E<sub>2</sub>, estradiol; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment of beta cell function; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triacylglycerol.



**Figure 1** Differences in metabolic variables of children across quarters of maternal E<sub>2</sub> levels stratified by embryo frozen Association between maternal E<sub>2</sub> and metabolic variables in offspring after Fresh (A) and Frozen (B) embryo transfer.

**Abbreviations:** FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triacylglycerol.

## Discussion

Our study indicated that children with higher maternal E<sub>2</sub> levels before conception manifested temporary increases in LDL-C levels during infancy. However, in childhood, those with different maternal E<sub>2</sub> levels before conception showed similar metabolic profiles. This indicates that maternal COH does not affect the long-term metabolic health of the children.

Ovarian stimulation was one of the necessary key procedures in IVF/ICSI treatment.<sup>19</sup> In a COH cycle of normal ovarian response, more than 5–8 mature follicles would develop and produce supraphysiological E<sub>2</sub> levels,<sup>11</sup> which is only 100–300 pg/mL in natural conceived women.<sup>20</sup> A previous study indicated that the elevation of steroid hormones could persist through the first trimester in women who underwent COH.<sup>21</sup> Maternal E<sub>2</sub> enters fetal circulation through the placenta.<sup>22</sup> Accumulating evidence suggests that children exposed to a higher maternal E<sub>2</sub> displayed poor neonatal outcomes,<sup>12,13</sup> deregulated cardiovascular functions,<sup>23</sup> and a less favorable thyroid hormone profile,<sup>24</sup> neurodevelopment,<sup>25,26</sup> and neonatal metabolism.<sup>15,16</sup>

**Table 3** Characteristics of Children Aged 2 to 3 Yrs

	Quartiles Based on Maternal E <sub>2</sub>				Total (n=2398)
	Q1(n=614) E <sub>2</sub> <2420	Q2(n=597) 2420≤E <sub>2</sub> <3839	Q3(n=618) 3839≤E <sub>2</sub> <5599	Q4(n=569) E <sub>2</sub> ≥5599	
<b>Perinatal characteristics</b>					
Birthweight, kg	3.47±0.55	3.46±0.52	3.44±0.50	3.45±0.49	3.45±0.52
Gestational age at birth, weeks	38.91±1.56	39.03±1.58	39.02±1.57	39.06±1.53	39.01±1.56
Sex (male), n (%)	335(54.6)	299(50.1)	352(57.0)	303(53.3)	1289(53.8)
<b>Metabolic variables</b>					
No. of visits	644	619	637	583	2483
BMI, kg/m <sup>2</sup>	15.88±1.46	15.92±1.51	15.91±1.45	15.91±1.47	15.90±1.47
FBG, mmol/l	4.87±0.39	4.89±0.39	4.86±0.39	4.89±0.41	4.87±0.40
Insulin, pmol/l	3.27(2.04, 4.90)	3.40(2.27, 5.03)	3.24(2.18, 4.90)	3.39(2.12, 4.91)	3.32(2.14, 4.93)
HOMA-IR	0.71(0.43, 1.10)	0.74(0.48, 1.12)	0.71(0.46, 1.09)	0.73(0.43, 1.08)	0.72(0.45, 1.09)
HOMA-β	50.81(34.13, 69.21)	50.57(36.48, 74.09)	49.00(34.50, 73.21)	50.54(35.78, 69.18)	50.21(34.97, 71.05)
TC, mmol/l	4.05±0.66	4.02±0.71	4.08±0.71	4.10±0.74	4.06±0.70
TG, mmol/l	0.73±0.33	0.74±0.29	0.73±0.29	0.74±0.28	0.73±0.30
LDL-C, mmol/l	2.42±0.55	2.41±0.59	2.45±0.59	2.49±0.60	2.44±0.58
HDL-C, mmol/l	1.40±0.29	1.39±0.30	1.40±0.30	1.38±0.30	1.39±0.30

**Notes:** Data are presented as the mean ± SD, median (interquartile range), or n (%).

**Abbreviations:** BMI, body mass index; E<sub>2</sub>, estradiol; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment of beta cell function; LDL-C, low-density lipoprotein cholesterol; TC=total cholesterol; TG, triacylglycerol.

In this study, we also found a temporary increase in LDL-C levels in children within the highest quartile of maternal E<sub>2</sub> levels during the infancy phase. This may be affected by their mothers, whose serum LDL-C, TG, and Apo-B levels were reported to increase after ovarian stimulation.<sup>26</sup> Intrauterine exposure to elevated maternal lipid levels may upregulate genes involved in de novo lipogenesis, cholesterol synthesis, and dysregulation of genes related to lipid metabolism in offspring.<sup>27,28</sup> In addition, increased E<sub>2</sub> levels after ovarian stimulation could occur throughout the gestation period in fresh cycles and could be distributed into the fetal bloodstream.<sup>16,23</sup> Furthermore, it was demonstrated that E<sub>2</sub> could stimulate the synthesis of LDL-C and TC via increased expression of the cholesterol synthesis rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in fetal livers,<sup>16</sup> which was supported by the increasing trend of LDL-C in fresh ET. Moreover, higher maternal E<sub>2</sub> levels indicate a higher dose of exogenous gonadotropin, which was suggested to affect the next generation through epigenetic modification and oxidative stress status of oocytes, embryos, and their microenvironment.<sup>29–34</sup> However, it should be noted that according to our data, the difference vanished in the later childhood phase. This suggests that adverse effects on neonates may be auto-adjusted with age.

The strength of our present study was the long duration of follow-up, which extended the confirmation of the safety of COH and subsequent E<sub>2</sub> elevation in the next generation, the large sample size, which provided preferably statistical power and allowed the subgroup analysis to confirm the robustness of the conclusions, and the high homogeneity of the participants. However, this study has several limitations. First, the ideal control should be offspring born after natural cycle ART. However, this was difficult to obtain because the natural cycle was most suitable for women with diminished ovarian reserve (DOR) or older age, which would be an important confounding factor. Second, the metabolic profiles examined in the present study were only general tests that could not sufficiently represent the metabolic status in detail. Metabolomic analysis should be confirmed in future studies.

## Conclusion

In conclusion, the elevation of E<sub>2</sub> levels after COH in mothers did not seem to affect their offspring in terms of metabolism, except for a temporary change in LDL-C levels during infancy. The long-term safety of COH in the next

**Table 4** Mean Differences (95% CI) for Metabolic Variables of Children Aged 2 to 3 Yrs Across Quartiles of Maternal E<sub>2</sub> Levels

		Quartiles Based on Maternal E <sub>2</sub>						
		Q1 (644 Visits) E <sub>2</sub> <2420	Q2 (619 Visits) 2420≤E <sub>2</sub> <3839	p value	Q3 (637 Visits) 3839≤E <sub>2</sub> <5599	p value	Q4 (583 Visits) E <sub>2</sub> ≥5599	p value
BMI, kg/m <sup>2</sup>	Crude model	Ref	0.05(−0.25, 0.35)	0.971	0.06(−0.23, 0.36)	0.944	0.07(−0.23, 0.37)	0.937
	Adjusted model	Ref	0.07(−0.21, 0.35)	0.912	0.16(−0.13, 0.44)	0.495	0.15(−0.15, 0.44)	0.569
FBG, mmol/l	Crude model	Ref	0.01(−0.05, 0.06)	0.986	−0.02(−0.07, 0.04)	0.877	0.01(−0.05, 0.07)	0.962
	Adjusted model	Ref	0.01(−0.04, 0.07)	0.909	−0.01(−0.06, 0.05)	0.979	0.03(−0.03, 0.08)	0.676
Insulin, pmol/l <sup>†</sup>	Crude model	Ref	0.08(−0.02, 0.17)	0.139	0(−0.09, 0.09)	0.999	0.04(−0.06, 0.13)	0.736
	Adjusted model	Ref	0.07(−0.02, 0.16)	0.188	0.01(−0.08, 0.10)	0.988	0.04(−0.05, 0.14)	0.631
HOMA-IR <sup>†</sup>	Crude model	Ref	0.08(−0.02, 0.18)	0.168	−0.01(−0.10, 0.09)	0.999	0.04(−0.06, 0.14)	0.744
	Adjusted model	Ref	0.07(−0.02, 0.17)	0.206	0.01(−0.09, 0.11)	0.994	0.05(−0.05, 0.15)	0.599
HOMA-β <sup>†</sup>	Crude model	Ref	0.07(−0.01, 0.15)	0.133	0.03(−0.05, 0.11)	0.796	0.03(−0.05, 0.12)	0.752
	Adjusted model	Ref	0.06(−0.02, 0.14)	0.247	0.04(−0.04, 0.12)	0.650	0.03(−0.06, 0.11)	0.825
TC, mmol/l	Crude model	Ref	−0.04(−0.14, 0.06)	0.753	0.01(−0.08, 0.11)	0.985	0.05(−0.05, 0.15)	0.561
	Adjusted model	Ref	−0.03(−0.13, 0.07)	0.887	0.04(−0.06, 0.14)	0.768	0.09(−0.02, 0.19)	0.135
TG, mmol/l	Crude model	Ref	0.01(−0.04, 0.05)	0.974	0.01(−0.04, 0.05)	0.988	0.01(−0.03, 0.06)	0.857
	Adjusted model	Ref	0(−0.04, 0.05)	0.999	0.01(−0.04, 0.05)	0.975	0.02(−0.03, 0.06)	0.819
LDL-C, mmol/l	Crude model	Ref	−0.02(−0.1, 0.06)	0.919	0.03(−0.05, 0.11)	0.793	0.07(−0.01, 0.15)	0.121
	Adjusted model	Ref	−0.01(−0.1, 0.07)	0.973	0.05(−0.03, 0.14)	0.345	<b>0.11(0.02, 0.20)</b>	<b>0.005*</b>
HDL-C, mmol/l	Crude model	Ref	−0.01(−0.05, 0.03)	0.895	0(−0.05, 0.04)	0.995	−0.03(−0.07, 0.01)	0.333
	Adjusted model	Ref	0(−0.05, 0.04)	0.994	0(−0.05, 0.04)	0.994	−0.03(−0.07, 0.01)	0.317

**Notes:** Mean differences were obtained using a linear mixed-effects regression model, \*P<0.05, in bold. <sup>†</sup>Loge-transformed insulin, HOMA-IR, and HOMA-β values were considered continuous variables. Adjusted model: adjusted for maternal age, BMI, paternal age, BMI, cycle type, number of embryos transfer, stage of embryo transfer, offspring gestational age, birth weight, sex and age at visit.

**Abbreviations:** BMI, body mass index; E<sub>2</sub>, estradiol; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment of beta cell function; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triacylglycerol.

generation was favorable, at least during childhood. However, the trend of gradient increase in LDL-C suggests the need for close monitoring in the later life of those born after fresh ET.

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## Disclosure

The authors report no conflicts of interest in this work.

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