

The effect of supraphysiological estradiol levels in ovarian stimulation cycles on the birthweight of neonates conceived through subsequent frozen-thawed cycles is modified by endometrial preparation protocols

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Abstract

Background

Supraphysiological estradiol (E2) levels during ovarian stimulation are associated with adverse neonatal outcomes after fresh cycle embryo transfer. While frozen-thawed embryo transfer (FET) mitigates fresh cycle risks, oocytes remain exposed to high E2. Conflicting evidence on effect of E2 on neonatal birthweight in FET cycles suggests heterogeneity, possibly from differing endometrial preparation protocols. This study investigated how hormone replacement therapy (HRT) versus natural cycle (NC) protocols modifies the effect of high E2 on singleton birthweight.

Method

This retrospective cohort study analyzed singleton live births from FET cycles between 2013 and 2022. Patients who delivered live-birth singleton in frozen-thawed embryo transfer cycles with HRT or NC endometrium preparation protocols were enrolled. Propensity score matching (PSM) was utilized to balance baseline characteristics between the groups and generalized additive models (GAM) were used to evaluate the neonatal outcomes.

Results

A total of 6,468 singleton live births were included for analysis and finally 6262 singleton live births were generated for comparison after propensity score matching. Birthweight z-scores decreased linearly with E2 in NC cycles, whereas an inverted U-shaped pattern occurred in HRT cycles (inflection point: 1096 pg/ml). Similarly, LGA was negatively associated with peak E2 in NC cycles (edf = 1, P = 0.003) but not in HRT cycles (edf = 2.9, P = 0.27). Interaction analysis confirmed that endometrial preparation protocols significantly modified the impact of peak E2 on neonatal outcomes.

Conclusion

The impact of supraphysiological E2 levels during COS on neonatal birthweight in subsequent FET cycles is significantly modified by the endometrial preparation protocol. Unlike the linear negative association between E2 and birth weight observed in NC cycles, the HRT protocol appeared to mitigate the adverse effects of high E2 exposure. However, for cases involving low E2, the NC protocol shows better neonatal outcomes. The choice of endometrial preparation protocol should be selected on an individualized basis.

Introduction

Supraphysiologic estradiol environment driven by multiple follicle growth is a significant concern during the assisted reproductive technology (ART) treatment. The excessive estradiol levels in patients undergoing ovarian stimulation are not only associated with the risk of ovarian hyperstimulation syndrome (OHSS), but may also create an unsatisfactory endometrial environment for implantation (1, 2), leading to adverse neonatal outcomes after fresh cycle embryo transfer, such as the increased risk of low birth weight (LBW) and small for gestational age (SGA) infants (3). To prevent these adverse effects, frozen embryo transfer (FET) cycles are often recommended to patients with excessively high estradiol levels. Nevertheless, the oocytes in these patients have already been exposed to high estradiol levels, although the embryos are transferred in FET cycles. It is supposed that estradiol exposure may affect the epigenetic programming of oocytes via mechanisms such as overexpression of TET2 protein (4). Several studies have linked the supraphysiological estradiol levels in ovarian stimulation cycles with the low birthweight of neonates conceived through subsequent frozen-thawed cycles (3, 5–7). However, the controversy remains, and there are also several large retrospective studies that failed to identify significant associations between estradiol level during ovarian stimulation and neonatal birthweight in freeze-all cycles (8–10). The conflicting evidence may result from several types of bias, heterogeneity, or confounders. Among them, the selection of endometrial preparation protocols may represent a significant source of confounding.

To implement FET treatment, various endometrial preparation protocols have been employed, among which the hormone replacement treatment (HRT) cycles and natural cycles (NC) are the most used. Despite that both methods result in similar live birth rates according to recent RCTs and meta-analysis (11–13), they may create distinct maternal endocrine environments that affect the neonatal health (14, 15). In particular, HRT cycles lacking a functional corpus luteum may be associated with a higher risk of gestational hypertension and placental-related complications, which in turn may have a potential impact on neonatal birth outcomes (14, 16, 17). The placental function is also a postulated target of epigenetic dysfunction that resulted from estradiol-exposed oocytes in high responders (4). The potential synergistic or antagonistic effects of these exposures during FET cycles, therefore, underscore the need to evaluate neonatal safety under different treatment combinations.

The present study aims to investigate the potential patterns and intensities of the interaction between high E2 levels in the COS cycles and different FET endometrial preparation protocols, as well as the combined effects of the two on the outcomes of singleton live neonates.

Materials and Methods

Study design and subjects

A retrospective cohort study was conducted at the Reproduction Medicine Center of Xiamen University Affiliated Chenggong Hospital. Patients who delivered live-birth singleton in frozen-thawed embryo transfer cycles with HRT or NC endometrium preparation protocols were enrolled from 2013 to 2022. Detailed workflow of inclusion and exclusion of patients is shown in supplementary **figure S1**. Information related to neonatal outcomes was gathered through a telephone survey. The study was approved by the Institutional Review Board of Xiamen University Affiliated Chenggong Hospital.

Ovarian stimulation and Endometrium preparation protocols

Agonist protocol and antagonist protocol were the two main COS protocols. The selection of COS protocol was mainly determined by patients' age, baseline hormone levels, antral follicle count (AFC), body mass index (BMI), and ovarian response as described (18). In all cases, intermittent vaginal ultrasound monitoring of follicle growth and blood tests for estradiol (E2), luteinizing hormone (LH), and progesterone (P) levels are performed. When two follicles have a diameter of ≥ 17 mm or one follicle has a diameter of ≥ 18 mm, the trigger is activated. The oocyte is retrieved 36 to 37 hours after the trigger.

The decision on endometrial preparation protocols depended on logistical reasons or preference. In NC cycles, a transvaginal ultrasound is performed from the 8th to the 12th day of menstruation to monitor the size of follicles and the thickness of the endometrium. When the diameter of the dominant follicle is ≤ 14 mm, monitoring should be conducted every 1–2 days. When the diameter is ≥ 16 mm, daily monitoring becomes a must, and a urine LH test strip detection should be given simultaneously. When the detection shows a strong positive result, blood should be drawn to test E2, LH, and P levels. For patients with normal ovulation, dydrogesterone (Duphaston, Abbott, Netherlands) should be taken orally starting from the ovulation day (Day 0) for the luteal support at a dose of 40 mg per day. For patients with abnormal ovulation, the same dose is administered starting from the day following the LH peak (Day 0). Embryo transfer is scheduled on day 4 (cleavage embryo) or day 6 (blastocyst). Luteal support is continued after embryo transfer.

For HRT cycles, oral administration of estradiol valerate at a dose of 4–6 mg/d is initiated on the 2nd or 3rd day of the menstrual cycle. From the 8th day, the endometrial thickness is monitored by ultrasound. If the endometrial thickness is ≥ 8 mm, the original dose is maintained. If the endometrial thickness is less than 8 mm, the dosage of estradiol valerate should be increased as appropriate, or transdermal estradiol valerate gel should be supplemented. The maximum oral dosage is 8 mg/d. Luteal support was initiated when estradiol valerate was taken for 14–16 days, and the endometrial thickness was ≥ 8 mm. Vaginal progesterone gel (Crinone, Merck, Germany) at 90 mg per day and oral dydrogesterone at 40 mg were administered. If the endometrial thickness is less than 7 mm after taking estradiol valerate for 14 to 16 days, the drug administration time should be appropriately extended.

Live birth and neonatal outcomes

A live birth in medically assisted reproduction is defined as a complete expulsion or extraction from its mother of a product of fertilization, irrespective of the duration of the pregnancy, which, after such separation, breathes or shows any other evidence of life, such as heart beat, umbilical cord pulsation, or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached (19).

The main neonatal outcomes of the study were about birthweight, including absolute birthweight, Z-score, HBW, LBW, LGA, and SGA. The definitions were based on a set of general population reference values for Chinese singletons (20, 21), Z-score was adopted for the standardization of birthweight after adjusting for gestational weeks and neonatal sex. HBW was defined as birthweight > 4000 g, LBW was defined as birthweight < 2500 g, LGA was defined as infants with birthweight > 90 th percentile, while infants with birthweight < 10 th percentile of the Chinese singleton population were categorized as SGA.

Statistics analysis

Based on the national reference data of neonatal weight, the z-score of birth weight adjusted for neonatal gender and gestational age was calculated. All estradiol data were subjected to natural logarithmic conversion ($\lg E2$) for further evaluation. We used the generalized additive model (GAM) in the R package ("mgcv") to evaluate the dose-response relationship between estradiol levels in the COS cycle and the z-score of birth weight in the HRT and NC cycles. Use the "tidymv" package to compare the differences between smooth curves. To minimize the confusion of evidence, we further verified the comparison results in the subgroups with propensity score matching. We used the generalized linear model (GLM) to estimate the effect of estradiol on the z-score of birth weight within the estradiol monotonic interval shown by GAM analysis. All covariates and confounding factors included in the above model were determined based on existing knowledge.

Results

A total of 6,468 singleton infants from HRT ($n=3335$) or NC ($n=3133$) FET cycles were included in our study. After calculating the propensity score, 3131 HRT cycles matched with 3131 NC cycles. Patients' clinical characteristics are shown in **Table 1**. After propensity matching, there were no significant differences between the HRT group and the NC group in terms of major covariates, such as female age, parity, height, weight, BMI, basal FSH/LH, as well as log transformed peak E2 ($D>0.1$). The distribution of propensity score was also identical between NC and HRT cycles after matching (**Figure S2**). Before and after matching, the HRT cycles were prone to have higher birthweight, increased birthweight z-score, and greater risks of LGA. However, there was no significant difference in SGA between the groups.

A general additive model (GAM) was carried out to detect the association between the birth weight of FET cycles and the peak E2 level in the COS cycles. In both matched and unmatched cycles, a dose-dependent effect of peak E2 level in the COS cycle and birthweight z-score in different endometrial preparation protocols was observed. A negative linear association in NC cycles was observed in both the matched ($\text{edf}=1.3$, $P=0.02$) and the unmatched cohort ($\text{edf}=1.2$, $P=0.02$). In contrast, an inverted U-shaped curve association in HRT cycles between Z-score and peak E2 were observed in the unmatched ($\text{edf}=2.8$, $P=0.09$) and matched ($\text{edf}=2.7$, $P=0.13$) cohort. A similar negative linear association was shown between LGA and peak E2. LGA was negatively associated with peak E2 in the NC cycles ($\text{edf}=1$, $P=0.03$ for both the unmatched and matched cohorts) but not in the HRT cycles ($\text{edf}=2.9$, $P=0.27$ in the unmatched cohort, $\text{edf}=3.1$, $P=0.31$ in the matched cohort) (**Figure 1 & Figure S3**).

To estimate the interaction between peak E2 and endometrial preparation in the smoothed splines, an interaction term was introduced, and the difference-in-spline was calculated using the "tidymv" package. It is noted that significant differences (pink area) were observed (**Figure 1**). When the peak E2 was less than 148 pg/ml ($\lg E2<5$), the birth weight z-score in HRT cycle was lower than that in NC cycles. Alternatively, the birth weight z-score in HRT cycle was significantly higher than those in NC cycles when the values of $\lg E2$ was between 7.527 (1857 pg/ml) and 9.139 (9331 pg/ml). A similar trend in difference

was also observed in the matched cohort and for the outcome of LGA, despite that the difference was no longer significant due to reduced power when $\lg E2 < 5$ (**Figure 1**).

According to the thresholds (7.527 and 9.139), the patients were stratified into three groups. When the logarithm of peak E2 level was between 7.527 and 9.139, the neonatal birthweight was significantly associated with the endometrial preparation protocol. The birthweight and Z-score, as well as, the incidence of LGA and HBW was higher in HRT cycles compared with NC cycles. At the same time, when the logarithm of peak E2 level was outside the range of greater than 7.527 and less than 9.139, the neonatal birthweight showed no difference in different endometrial preparation protocols (**Table S1**). A generalized linear model (GLM) suggested an interaction with marginal P value (0.04) when $\log E2 \leq 7.527$, suggesting that NC cycles might reduce the effect size of peak E2 exposure by 0.48(95%CI: -0.95, -0.01) in comparison with HRT cycles. In contrast, the interaction term was insignificant when E2 level was in higher category.

The difference-in-spline curve converged the x axis at $\lg E2$ of 7, where the difference of HRT and NC changed from negative to positive (**Figure 1**). When 7 was chosen as the threshold, GLM analysis showed that HRT cycles were prone to have lower z-scores when the estradiol levels of COS cycles were lower ($\lg E2 < 7$), but led to higher z-scores when the estradiol levels increased (**Table 2**). An interaction analysis in a multivariate model suggested a significant modification of estradiol thresholds on the difference between NC and HRT cycles on birthweight z-score (estimate 0.39, 95%CI: 0.13-0.64, $P=0.003$). It indicated that the difference between HRT and NC cycles in birthweight z-score would increase by 0.39 if the peak estradiol levels in the COS cycle exceeded 1096 pg/ml.

Discussion

Main Finding

Through a large-sample retrospective analysis, this study revealed a significant interaction between the endometrial preparation protocol (HRT vs NC) and the E2 level in the COS cycle, jointly affecting the birth weight of neonates. While a clear linear association between E2 and birthweight z-score was observed in NC cycles, the association in HRT cycles was inverted U-shaped. The GAM model suggests an E2 level of 1096 pg/ml as an inflection point of the interaction. When the estradiol levels were lower than the point, the z-score in NC cycles was higher than that in HRT cycles. In contrast, HRT cycles led to a higher z-score and LGA rate in patients with higher estradiol levels in COS cycles. The data suggested that the effect of HRT might mask the association between peak estradiol levels in COS cycles and neonatal outcomes following FET, especially when cycles with lower estradiol levels were used as the reference category. In addition, the lower birthweight in HRT cycles in comparison with NC cycles among patients with estradiol levels lower than 1000 pg/ml also supported the concept of using the NC cycle if possible, even though the HRT is usually used for logistical reasons.

Interpretation

A number of previous studies, including our own, have evaluated the association between the peak E2 level of COS cycles and neonatal outcomes following FET cycles, yielding conflicting conclusions (1, 7, 8, 22). For instance, Duan et al. reviewed 10581 FET cycles, demonstrating that higher E2 level during COS was associated with higher incidence of preterm birth and SGA among singletons following FET (7). In contrast, Huang et al. categorized 8501 singleton live births from first freeze-all IVF/ICSI cycles into six groups according to the E2 levels on trigger day and no significant differences among the six groups (9). The disparity could be attributed to various factors, such as population heterogeneity and unknown confounding. Importantly, the difference in the distribution of endometrial preparation protocols is also noted. In the study of Duan et al, approximately 44% of the cycles were prepared in NC cycles (7). In the work of Huang et al., the proportion of NC cycles was only 29% (9). As previous studies are based on multiple comparisons between E2 categories (7, 9), the uneven distribution of endometrial preparation protocols across categories would skew the statistical significance of the comparison. Although Huang et al. failed to show a significant association between peak E2 and birthweight following FET, their data in NC cycles also suggested a negative mean difference comparing higher E2 categories with the reference category (9).

Notably, the distribution of endometrial preparation protocols may also indicate selection bias of patients despite the efficacy of NC and HRT cycle is similar according to RCTs (12). In several studies (3, 9), the HRT cycles were assigned to patients with irregular menstrual cycles, and stimulated cycles were assigned to patients with a history of thin endometrium. The irregular menstrual cycle is known to link with diagnoses such as PCOS, which may affect the maternal metabolism and neonatal outcomes (23, 24). On the other hand, a thin endometrium may be associated with a lower offspring birthweight in FET cycles (25). Including such cases may increase the uncertainty of the analyses and skew the conclusion. For these reasons, we excluded the PCOS patients and patients with stimulation cycles.

The endometrium preparation protocol is known to be an important determinant of neonatal outcomes in FET cycles (26–28). With the absence of corpus luteum, HRT cycles have been associated with poorer neonatal outcomes compared with NC cycles (14, 16, 17). Recent meta-analysis comparing the obstetric and neonatal outcomes following NC-FET and HRT-FET cycles have shown that the average neonatal birthweight as well as the risks of LGA was higher following HRT-FET compared to NC-FET (29, 30). However, increased risks of low birth weight were also reported in singletons following HRT cycles compared with NC cycles (14). In general, our data comply with the previous findings. However, it is also noted that neonates following NC cycles appeared to have higher birthweight z-score compared with HRT cycles when the peak E2 levels in COS cycles were lower than 1096 pg/ml, suggesting that the poor responders may have babies with lower birthweight in HRT cycles. Interestingly, a previous study has shown that elevated basal FSH level, a well-known predictor for poor response, is a positive predictor for LBW with an AUC of 0.646 (31). Sabatini et al. postulated that patients with a high value of basal FSH might tend to receive an increased dose of gonadotropin, which would lead to a suboptimal developmental potential of embryos in subsequent FET cycles (32). Unlike supraphysiological estradiol exposure, which might lead to TET overexpression and DNA hypomethylation (4), high gonadotropin resulted in dose-response hypermethylation in several DMRs, such as *Gfod2*, which may play roles in the metabolism of the offspring (33). In addition, predicted poor responders are also arguably associated with poor oocyte quality (34, 35). In our data, the decreased birthweight z-score in patients with low estradiol levels

might imply an interaction between the specific type of patients and the selection of the endometrial preparation protocols. It may also provide a potential explanation for the coexistence of increased incidence of LBW and LGA in the reported HRT cohorts (29, 30).

The interaction between peak estradiol levels in COS and endometrial preparation protocols also suggested that HRT cycles may mask the effect of previous estradiol exposure on birthweight. However, it does not necessarily mean that the effect of estradiol exposure is neutralized in the HRT cycles. While birthweight is a surrogate marker of growth trajectory, it is codetermined by the embryos and maternal physiology. Although the HRT cycles may result in an overwhelming maternal endocrine environment during early pregnancy, the imprinting consequence of COS exposure, such as the hypomethylated MEST genes (4), might not diminish. The possibility that the epigenetic abnormalities associated with the exposure warrant further follow-up studies.

Strengths and limitations

The strengths of the study included a relatively large number of subject as well as a GAM model capturing the nonlinear dose-dependent response relationship with continuous variables. In almost all previous studies (7, 9), continuous estradiol levels were categorized, compared to the lowest category, which raised concerns regarding reducing statistical power, multiple comparisons, and heterogeneity within categories (36). In combination with GLM the GAM model would provide a more flexible and robust estimate of the associations, identifying potential inflection points of the effects. However, this study is also limited by its retrospective nature and the single-center design. A retrospective design cannot eliminate unmeasured residual confounding factors. Secondly, the lack of detailed data on embryo quality and molecular biological indicators of placental tissue limits in-depth exploration of potential mechanisms (such as epigenetic alterations). Single-center data and the lack of long-term follow-up on the metabolic health of the offspring children limited the extrapolation of the conclusion. Multi-center prospective cohort studies are needed for further verification. In addition, the sample size of patients with lower estradiol levels during the COS cycle was limited, which reduced the statistical power and increased uncertainty.

Conclusion

The endometrial preparation protocol significantly modifies the impact of E2 levels in the COS cycle on the birth weight of neonates. Compared with the NC cycle, the HRT cycle may have a significant interaction with the peak E2 level of the COS cycle. The HRT protocol may alleviate the negative impact of high E2 to some extent, but in the case of low E2, the NC protocol shows better neonatal outcomes. Therefore, in clinical practice, the FET endometrial preparation protocol should be selected on an individualized basis. This not only concerns the success rate of pregnancy but also is of great significance for optimizing the short-term and long-term health outcomes of the offspring. Future research should focus on integrating multi-omics techniques to clarify the specific molecular pathways by which E2 affects embryonic and placental development, and conduct long-term follow-up studies on offspring.

Declarations

Ethical approval

The study was approved by the Ethics Committee of Xiamen University Affiliated Chenggong Hospital (Approval number: 73JYY2026223282). This study was conducted in accordance with the principles of the Declaration of Helsinki.

Consent to participate

Informed consent was unnecessary because the research was based on non-identifiable records approved by the Ethics Committee of Xiamen University Affiliated Chenggong Hospital.

Consent to publish

Not Applicable.

Competing interests

None declare.

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Author Contribution

J.G., J.C., and X.J. contribute to conception and design. J.G., C.M., and J.R. contribute to the acquisition of data. Y.C., J.G., H.C., L.L., and Z.L. contribute to the experimental sample collection. J.G., J.C., Y.C., and L.O. contribute to the analysis and interpretation of data. All authors contributed to drafting the article or revising it critically for important intellectual content. All authors read and approved the final manuscript.

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Data Availability

The data that support the findings of this study were provided by the Affiliated Chenggong Hospital of Xiamen University by permission. Data will be shared on request to the corresponding author with the permission of the Affiliated Chenggong Hospital of Xiamen University.

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Tables

Table 1 Clinic characteristics of HRT and NC patients before and after ps-matching.

	Unmatched			Matched
	HRT	NC	P-value	HRT
	(N=3335)	(N=3133)		(N=3131)
Female Age				
Mean(SD)	31.4(3.97)	31.4(3.83)	0.992	31.5(3.98)
Median[Q1,Q3]	31.0[29.0,34.0]	31.0[29.0,34.0]		31.0[29.0,34.0]
Parity				
Mean(SD)	0.292(0.525)	0.297(0.535)	0.884	0.298(0.530)
Median[Q1,Q3]	0[0,1.00]	0[0,1.00]		0[0,1.00]
Height				
Mean(SD)	158(5.20)	158(5.10)	0.034	158(5.14)
Median[Q1,Q3]	158[155,162]	158[155,162]		158[155,162]
Weight				
Mean(SD)	52.3(6.23)	52.4(6.01)	0.436	52.4(6.20)
Median[Q1,Q3]	52.0[48.0,56.0]	52.0[48.0,56.0]		52.0[48.0,56.0]
BMI				
Mean(SD)	20.9(2.12)	20.8(2.11)	0.284	20.9(2.13)
Median[Q1,Q3]	20.9[19.3,22.5]	20.8[19.3,22.4]		20.9[19.4,22.5]
bFSH				
Mean(SD)	7.09(2.25)	7.16(2.21)	0.026	7.08(2.18)
Median[Q1,Q3]	6.74[5.78,7.97]	6.86[5.88,8.01]		6.74[5.78,7.93]
bLH				
Mean(SD)	6.81(92.9)	4.81(2.76)	<0.001	4.84(2.47)
Median[Q1,Q3]	4.55[3.44,6.03]	4.36[3.35,5.65]		4.44[3.39,5.76]
IgE2				
Mean(SD)	8.26(0.676)	8.33(0.630)	<0.001	8.28(0.662)
Median[Q1,Q3]	8.36[7.86,8.73]	8.42[7.96,8.76]		8.37[7.89,8.74]
ICSI	910 (27.3%)	917 (29.3%)	0.081	867 (27.7%)
BT	2947 (88.4%)	2730 (87.1%)	0.142	2759 (88.1%)
Oocyte				
Mean(SD)	12.6(6.25)	13.6(6.40)	<0.001	12.9(6.27)
Median[Q1,Q3]	12.0[8.00,16.0]	13.0[9.00,17.0]		12.0[8.00,17.0]
Birthweight				
Mean(SD)	3290(496)	3260(449)	<0.001	3290(494)
Median[Q1,Q3]	3300[3000,3600]	3300[3000,3550]		3300[3000,3600]
GA				
Mean(SD)	39.0(1.64)	39.0(1.46)	0.051	39.0(1.63)
Median[Q1,Q3]	39.0[38.0,40.0]	39.0[38.0,40.0]		39.0[38.0,40.0]
Z				
Mean(SD)	0.117(1.05)	0.015(1.01)	<0.001	0.119(1.05)
Median[Q1,Q3]	0.0995[-0.537,0.721]	0.0153[-0.622,0.578]		0.0995[-0.536,0.7
LGA	214 (6.4%)	146 (4.7%)	0.002	201 (6.4%)
SGA	146 (4.4%)	122 (3.9%)	0.361	136 (4.3%)

Note: HRT, hormone replacement treatment; NC, natural cycle; BMI, body mass index; ICSI, intracytoplasmic sperm injection; BT, blastocyst transfer; GA, ges

Table 2 The difference in birthweight among different endometrial preparation protocols in groups with different E2 levels.

	IgE2<=7			IgE2>7
	HRT	NC	P-value	HRT
	(N=168)	(N=104)		(N=3167)
Birthweight				
Mean(SD)	3270(482)	3360(440)	0.156	3290(497)
Median[Q1,Q3]	3300[3040,3600]	3430[3050,3650]		3300[3000,3600]
Z				
Mean(SD)	0.0878(0.962)	0.361(1.03)	0.059	0.119(1.05)
Median[Q1,Q3]	0.0711[-0.487,0.796]	0.316[-0.227,0.882]		0.0995[-0.546,0.721]
GA				
Mean(SD)	38.9(1.67)	38.8(1.72)	0.741	39.0(1.64)
Median[Q1,Q3]	39.0[38.0,40.0]	39.0[38.0,40.0]		39.0[38.0,40.0]
Sex				
female	72 (42.9%)	40 (38.5%)	0.556	1440 (45.5%)
male	96 (57.1%)	64 (61.5%)		1727 (54.5%)
SGA	6 (3.6%)	1 (1.0%)	0.354	140 (4.4%)
LGA	7 (4.2%)	7 (6.7%)	0.517	207 (6.5%)
LBW	10 (6.0%)	3 (2.9%)	0.390	158 (5.0%)
HBW	3 (1.8%)	4 (3.8%)	0.516	155 (4.9%)
Adjusted difference in Z-score*	Ref	0.25(0.00, 0.49)	0.047	Ref

Note: HRT, hormone replacement treatment; NC, natural cycle; Z, z-score; GA, gestational age; SGA, small for gestational age; LGA, large for gestational age;

Figures

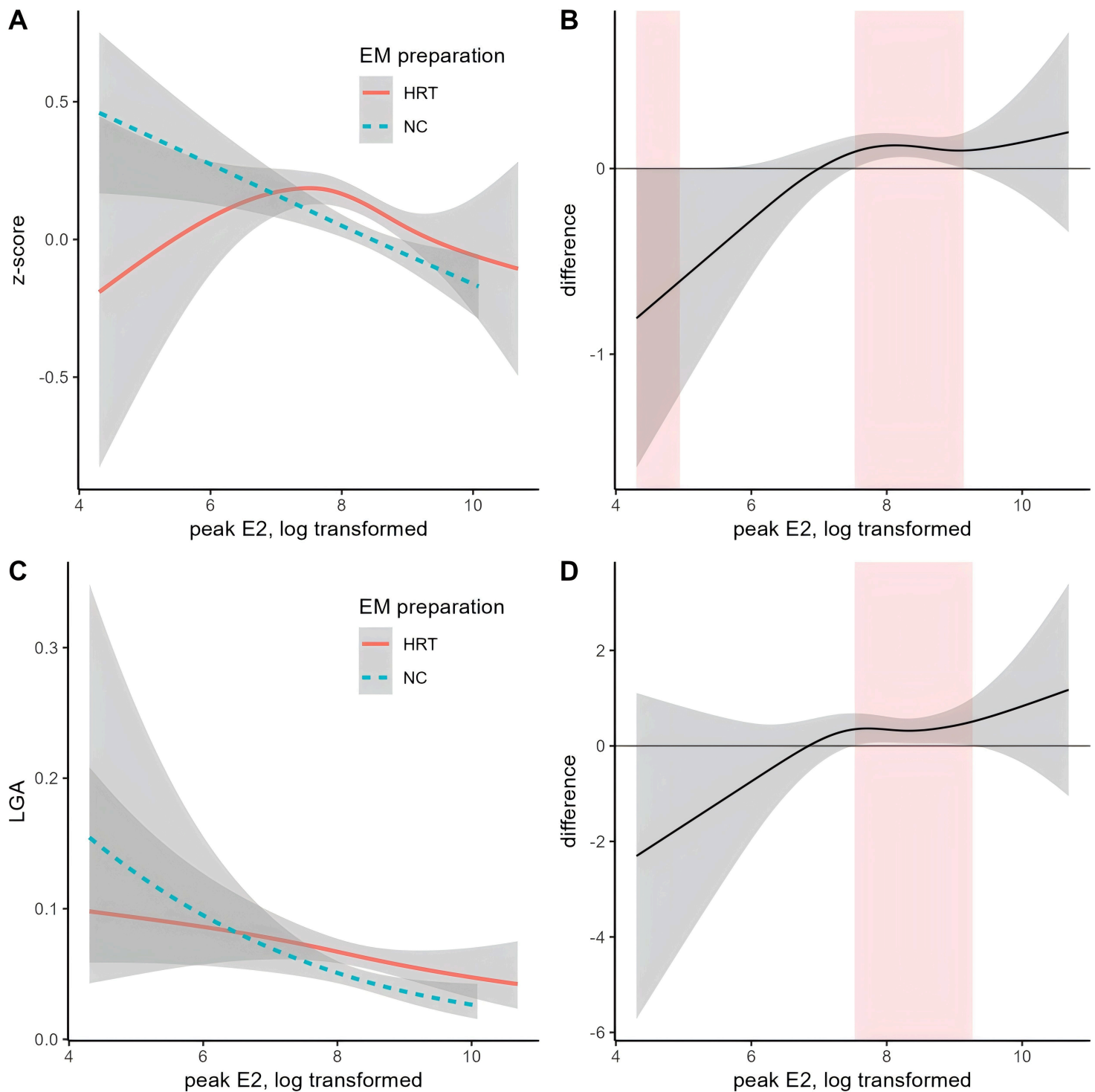


Figure 1

Association between peak estradiol levels during ovarian stimulation and birth weight of singletons following frozen-thawed embryo transfer cycles using different endometrial preparation protocols in the unmatched cycles. (A) Association between peak estradiol levels during ovarian stimulation and birth weight z-score of singleton following frozen-thawed embryo transfer (FET) cycles using natural (NC) and hormone replacement (HRT) cycles. (B) The difference in the association between peak estradiol levels during ovarian stimulation and birth weight z-score comparing singletons following HRT cycles with those following NC cycles. (C) Association between peak estradiol levels during ovarian stimulation and large-for-gestational-age (LGA) in singleton following FET cycles using NC and HRT cycles. (D) The difference in the association between peak estradiol levels during ovarian stimulation and FET comparing singletons following HRT cycles with those following NC cycles. The pink shaded area indicates a significant difference.

Supplementary Files

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- [supplementalBMC.docx](#)
- [TableS1.docx](#)