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Endometrial thickness is an independent risk factor of hypertensive disorders of pregnancy: a retrospective study of 13,458 patients in frozen-thawed embryo transfers

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Abstract

Background: Hypertensive disorders of pregnancy (HDP) are an important cause of maternal and fetal mortality, and its potential risk factors are still being explored. Endometrial thickness (EMT), as one of the important monitoring indicators of endometrial receptivity, has been confirmed to be related to the incidence of HDP in fresh embryo transfer. Our study was designed to investigate whether endometrial thickness is associated with the risk of hypertensive disorders of pregnancy in frozen-thawed embryo transfer (FET).

Methods: This respective cohort study enrolled 13,458 women who received vitrified embryo transfer and had a singleton delivery in the Reproductive Hospital affiliated to Shandong University from January 2015 to December 2019. We set strict screening criteria and obtained the information from the hospital electronic medical system. Statistical methods including logistic regression analysis, receiver operating characteristic curve and restricted cubic spline were used to evaluate the relationship between endometrial thickness and the incidence of pregnancy-induced hypertension.

Results: The incidences of HDP in a thin endometrial thickness group (<0.8 cm) and a thick endometrial thickness group (>1.2 cm) were significantly greater than in a reference group (0.8 cm–1.2 cm) (7.98 and 5.24% vs 4.59%, $P < 0.001$). A nonlinear relationship between endometrial thickness and risk of hypertensive disorders of pregnancy was examined by restricted cubic spline ($P < 0.001$). The thin endometrial thickness and thick endometrial thickness groups were significantly associated with the risk of HDP after adjusting for confounding variables by stepwise logistic regression analysis. Subsequently, subgroup logistic regression analysis based on endometrial preparation regimens showed that thin endometria were still significantly associated with a higher morbidity rate in the artificial cycle group, while in the natural cycle group, thick endometria were closely associated with increased morbidity.

Conclusion: Our study manifested that both the thin and thick endometria were associated with an increased risk of hypertensive disorders of pregnancy in frozen embryo transfer cycles. Reproductive clinicians should focus on adjusting endometrial thickness in different preparation regimens; and obstetricians should be mindful of the risk

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of hypertension during pregnancy, when women with thin (<0.8 cm) or excessively thicker (> 1.2 cm) endometrial thickness achieve pregnancy through frozen-thawed embryo transfer.

Keywords: IVF/ICSI, FET, Ultrasound measurements of endometrium thickness, Natural cycles, Hormone programmed cycles, Obstetric complications

Background

With refinements in assisted reproductive technology (ART), births following in vitro fertilization (IVF) have increased dramatically over the last two decades [1, 2]. Therefore, a higher pregnancy rate is no longer the focus of research; attention has turned to better obstetric and perinatal outcomes [3]. However, compared with spontaneous conception, pregnancies conceived by ART are associated with increased risk of obstetric complications and lower birth weight [4]. Now, several candidate risk factors including multiple pregnancy, hormonal stimulation, embryo culture and biopsy, and fresh/frozen embryo transfer (ET) policy have been revealed [5–7].

Frozen embryo transfer (FET) could increase the accumulated chance of pregnancy for patients with surplus embryos, and facilitate delayed transfer in cases of premature progesterone rise, ovarian hyper-stimulation syndrome, or preimplantation genetic screening after blastocyst biopsy [8–10]. Although a higher clinical pregnancy rate, decreased risks of small for gestational age, low birth weight, and preterm delivery in FET cycles have been reported over those of fresh ET, evidence from two recent meta-analyses shows some adverse obstetrics and perinatal outcomes after FET including pregnancy-induced hypertension, large for gestational age (LGA), and postpartum hemorrhage [3, 11]. These results indicate that FET and fresh ET have their respective advantages and disadvantages in obstetric and perinatal outcomes. Further studies have investigated the possible risk factors within fresh ET or FET cycles including high estrogen level, absence of a corpus luteum (CL), and thin endometrial thickness [12–15]. However, reasons for the impact of FET procedures on obstetrics and perinatal outcome require further study.

EMT is a key factor influencing endometrial receptivity and is a predictor of early pregnancy outcomes in ET cycles [16]. Furthermore, our previous studies demonstrate that thin EMT is associated with small for gestational age infants and hypertensive disorders of pregnancy in fresh ET cycles [17, 18]. Another study from our clinic shows that women with a singleton delivery after FET in artificial cycles had an elevated risk of pre-eclampsia and postpartum hemorrhage compared with those in natural cycles [19]. Therefore, the associations between EMT and obstetrics/perinatal outcomes

after FET with different endometrial preparation methods require urgent analysis.

The aim of this study was to investigate the association between EMT and the incidence of hypertensive disorders in singleton deliveries following the transfer of vitrified embryos with programmed or natural endometrial preparation cycles in IVF/ICSI (in vitro fertilization/intracytoplasmic sperm injection).

Methods

Study design and patients

This was a retrospective cohort study undertaken in the Reproductive Hospital of Shandong University, to investigate the effect of EMT on the risk of hypertensive diseases of pregnancy (HDP). The study was approved by the Ethics Committee of Reproductive Medicine of Shandong University. Inclusion criteria: (1) patients who were ≤ 40 y and ≥ 20 y (both oocyte retrieval age and embryo transfer age); (2) delivered a singleton infant after 28 weeks gestation; (3) systolic blood pressure < 140 mmHg; diastolic blood pressure < 90 mmHg before FET. Exclusion criteria: (1) preimplantation genetic testing; (2) pregnancies using donor sperm or donor oocyte; (3) chronic hypertension diseases or diabetes mellitus; (4) multiple birth; (5) fetal reduction; (6) vanishing twin syndrome after 14 weeks of gestation [20]; (7) uterine malformations. All basic data were collected from the hospital electronic medical system. Almost all the patients had hysteroscopic examination before their embryo transfer cycles. Women with moderate to severe intrauterine adhesion would accept hysteroscopic surgery to improve their intrauterine environment. Fifty-eight patients were lost to follow-up and two patients lacked vital information and were excluded. Finally, 13,458 patients were selected for the study.

Natural cycles/hormone programmed cycles

For natural cycles, follicles were monitored by transvaginal ultrasonography from day 8 to 10 day of the menstrual cycle. Serum or urine luteinizing hormone, and serum progesterone and estrogen were also tested to evaluate the exact ovulation time. Ovulation occurred either spontaneously or was induced by human chorionic gonadotropin. After ovulation was confirmed, the optimal recovery and transfer time of the vitrified embryos was determined according to frozen embryo stage.

For hormone programmed cycles, patients began taking 4–8 mg of oral estradiol for at least 10 days starting from day 2–5 of the menstrual cycle. If EMT was less than 0.8 cm, the estradiol dose could be increased and/or the duration of administration could be extended, until EMT reached 0.8 cm, or was <0.8 cm while the clinicians and the patients recognized it as a relatively maximum thickness; or the clinicians and the patients decided to terminate the current cycle. Then, progesterone would be given to start endometrial transformation.

Endometrium thickness assessment

For natural cycles, assessment of endometrial thickness was performed on the day that we could decide the (theoretical) ovulation day and the transfer day. While in programming cycles, assessment would take place before starting progesterone support. The maximal distance from one interface of the endometrium-myometrium to the other in the midsagittal plane of the uterus including the cervical canal was measured by ultrasound (GE medical systems, Co., Ltd). For each patient, the endometrium measurement was routinely carried by two ultrasound technicians, one was operator, the other was recorder and checker. And the same ultrasound images were real-time shared by the two technicians during the measurement to prevent operation or measure mistakes. Lastly, the measurements were recorded in the electronic medical system.

Patients' follow-up

As mentioned in a previous study [18], the first follow-up was performed around 14 d after FET, and biochemical pregnancy was assessed by measuring the serum HCG-beta subunit. The second follow-up was performed at 5 or 6 weeks after embryo transfer; a clinical pregnancy was confirmed by the presence of a gestational sac by transvaginal ultrasound. An ongoing pregnancy was confirmed at the third follow-up, which was performed at 12th week of gestation (9–10 weeks after ET). Subsequently, the patients would receive telephone surveys and standardized questionnaires by trained nurses. Information would be collected including perinatal complications, gestational weeks, birth date, delivery mode, newborn gender and birth weight, neonatal diseases, treatment, and prognosis. All follow-up information was recorded in the electronic medical records.

The target outcome was HDP, a disease that pregnancy coexists with elevated blood pressure, including gestational hypertension, pre-eclampsia, eclampsia, chronic hypertension complicating pre-eclampsia, as well as chronic hypertension complicating pregnancy. Our study covered only the first three categories. Gestational hypertension is diagnosed as: blood pressure

elevated $\geq 140/90$ mmHg after two or more measurements at least 4 h apart in pregnant women with previously normal blood pressure. Pre-eclampsia suggests that newly-systemic complications emerged coexistent with gestational hypertension, including proteinuria (protein ≥ 300 mg/24 h or a protein-to-creatinine ratio ≥ 0.30), blurred vision, and liver and kidney dysfunction. Moreover, eclampsia is both the onset of convulsions in a pregnant woman with pre-eclampsia without other causative conditions, and also a severe manifestation for HDP [21].

Statistical analysis

Initially, we performed descriptive statistics and univariate analyses with variables that may influence patient perinatal outcomes according to clinical experience and up-to-date literature. Continuous variables were presented as mean \pm standard deviation with one-way analysis of variance (ANOVA) and the between-groups differences were analyzed by LSD post-hoc multiple comparison. Categorical variables were expressed as frequencies and percentages, and the distribution among groups was analyzed by chi-square test or Fisher's exact test. Secondly, univariate and multivariate regression analyses were used to identify factors associated with the incidence of HDP. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to show the relationship between EMT and the risk of HDP after adjusting variables in multivariate regression analyses. All variates in multivariate regression analyses, including maternal age, body mass index (BMI), type of infertility, polycystic ovary syndrome (PCOS), history of intrauterine adhesion, previous birth, number of abortions, EMT, mean arterial pressure (MAP), development of embryos, gestational diabetes mellitus (GDM), cesarean section experience, and the number of embryos. PCOS was defined as a syndrome of ovarian dysfunction with clinical manifestations including menstrual irregularities, signs of androgen excess, and obesity according to the Rotterdam Consensus [22, 23]. Systolic blood pressure, diastolic blood pressure, and BMI, as general parameters, were measured before FET to estimate each patient's physical condition. Mean arterial pressure was an important index for hemodynamics [24]; it was equal to one-third of the sum of systolic blood pressure plus two-fold diastolic blood pressure in clinical work [25]. GDM could be diagnosis with a 75 g oral glucose tolerance test (OGTT) with one or more existing characteristics including fasting plasma glucose ≥ 5.1 mmol/L, a 1 h plasma glucose value ≥ 10.0 mmol/L, and a 2 h plasma glucose value ≥ 8.5 mmol/L [26]. *P*-values of <0.05 were considered statistically significant. Discrimination performance was illustrated by a receiver operating characteristic (ROC curve) using the area under the curve (AUC). Restricted

cubic spline was used to visualize the relation of predicted EMT with HDP incidence, and an EMT = 0.8 cm was set as the reference value. All statistical analyses were performed by using R v4.1.2.

Results

A total of 13,458 patients were enrolled for this research, consisting of 827 patients with an EMT < 0.8 cm, 1069 patients with an EMT > 1.2 cm, and 11,562 patients with

Table 1 Baseline demographic and clinical characteristics of patients

Variables	Normal EMT	Thin EMT	Thick EMT	P-values			
				All	Normal vs thin	Normal vs Thick	Thin vs Thick
Number	11,562	827	1069				
Maternal age (y)	30.41 (3.97)	31.05 (4.11)	30.51 (3.94)	< 0.001	< 0.001	0.473	0.003
Maternal BMI (kg/m²)	23.20 (3.51)	22.87 (3.34)	23.25 (3.42)	0.027	0.010	0.611	0.018
Paternal age (y)	31.12 (4.59)	31.76 (5.05)	30.87 (4.49)	< 0.001	< 0.001	0.096	< 0.001
Paternal BMI (kg/m²)	25.81 (4.08)	25.88 (4.19)	26.12 (4.14)	0.058	0.618	0.018	0.214
Type of infertility, n (%)							
Primary	6365 (55.05)	302 (36.52)	665 (62.21)				
Secondary	5197 (44.95)	525 (63.48)	404 (37.79)	< 0.001	< 0.001	< 0.001	< 0.001
PCOS, n (%)	2034 (17.59)	179 (21.64)	97 (9.07)	< 0.001	< 0.001	< 0.001	< 0.001
Intrauterine adhesion, n (%)	214 (1.85)	85 (10.28)	4 (0.37)	< 0.001	< 0.001	0.002	< 0.001
Childbirth experience, n (%)							
Nullipara	8127 (70.29)	551 (66.63)	726 (67.91)				
Pluripara	3435 (29.71)	276 (33.37)	343 (32.09)	0.029	0.087	0.336	0.999
Number of abortions, n (%)							
0	7545 (65.26)	374 (45.22)	749 (70.07)				
1	2825 (24.43)	263 (31.80)	220 (20.58)				
≥ 2	1192 (10.31)	190 (22.97)	100 (9.35)	< 0.001	< 0.001	0.017	< 0.001
Development of embryo, n (%)							
Blastocyst	11,367 (98.31)	820 (99.15)	1049 (98.13)				
Cleavage-stage	195 (1.69)	7 (0.85)	20 (1.87)	0.157	0.267	0.999	0.284
Mean EMT (cm)	0.97 (0.12)	0.72 (0.05)	1.32 (0.08)	< 0.001	< 0.001	< 0.001	< 0.001
Delivery model, n (%)							
Spontaneous labor	3567 (30.85)	210 (25.39)	381 (35.64)				
Cesarean labor	7995 (69.15)	617 (74.61)	688 (64.36)	< 0.001	0.003	0.004	< 0.001
Mean neonatal weight (kg)	3.46 (0.51)	3.38 (0.56)	3.45 (0.5)	< 0.001	< 0.001	0.587	0.005
Outcome, n (%)							
Full-term birth	10,825 (93.63)	758 (91.66)	1007 (94.20)				
Pre-term birth	704 (6.09)	66 (7.98)	59 (5.52)				
Post-term birth	33 (0.29)	3 (0.36)	3 (0.28)	0.215	0.256	0.999	0.285
HDP, n (%)	531 (4.59)	66 (7.98)	56 (5.24)	< 0.001	< 0.001	0.999	0.061
Number of embryos, n (%)							
1	10,652 (92.13)	774 (93.59)	1000 (93.55)				
> 1	910 (7.87)	53 (6.41)	69 (6.45)	0.093	0.454	0.343	0.999
History of cesarean delivery, n (%)	1724 (14.91)	127 (15.36)	155 (14.50)	0.873	0.999	0.999	0.999
Endometrial preparation regimen, n (%)							
Natural	7190 (62.19)	350 (42.32)	916 (85.69)				
Artificial	4372 (37.81)	477 (57.68)	153 (14.31)	< 0.001	< 0.001	< 0.001	< 0.001
GDM, n (%)	836 (7.23)	71 (8.59)	84 (7.86)	0.288	0.507	0.999	0.999
Systolic blood pressure (mmHg)	116.54 (11.89)	115.46 (11.54)	117.09 (11.60)	0.011	0.012	0.146	0.003
Diastolic blood pressure (mmHg)	69.66 (8.68)	68.94 (8.41)	70.12 (8.87)	0.013	0.020	0.102	0.003

Values are presented as mean ± standard deviation or n (%)

The between-groups differences in continuous variables are analyzed by LSD post-hoc multiple comparison

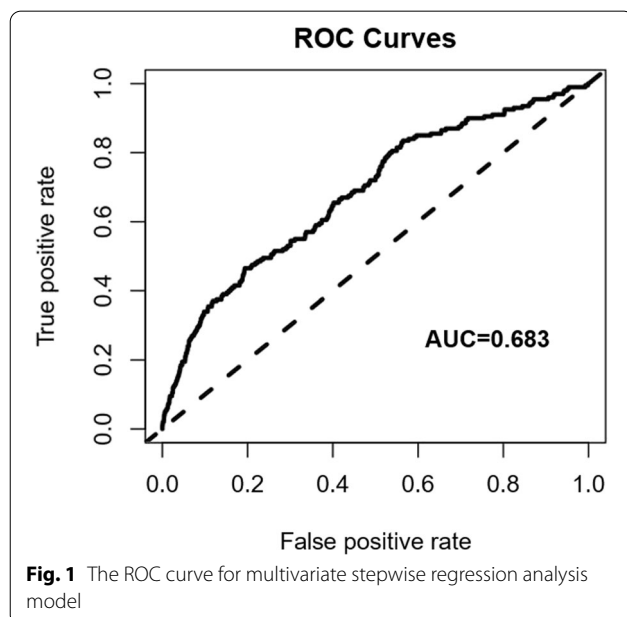
BMI Body mass index, *EMT* Endometrial thickness, *PCOS* Polycystic ovary syndrome, *HDP* Hypertensive disorders of pregnancy, *GDM* Gestational diabetes mellitus

Table 2 Logistic regression analysis of predictor variables for hypertensive disorders of pregnancy

Variable	Univariate logistic regression		Multivariate logistic regression		Stepwise logistic regression	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Maternal age	1.02 (0.94–1.10)	0.602	1.10 (1.01–1.20)	0.035	1.11 (1.02–1.21)	0.022
Maternal BMI	1.53 (1.42–1.64)	<0.001	1.30 (1.21–1.40)	<0.001	1.29 (1.20–1.39)	<0.001
MAP	1.78 (1.64–1.94)	<0.001	1.69 (1.55–1.84)	<0.001	1.68 (1.54–1.83)	<0.001
GDM	2.17 (1.71–2.72)	<0.001	1.82 (1.42–2.30)	<0.001	1.79 (1.40–2.27)	<0.001
Type of infertility	0.94 (0.80–1.10)	0.457	1.12 (0.90–1.39)	0.303		
PCOS	1.77 (1.47–2.11)	<0.001	0.91 (0.74–1.12)	0.378		
Intrauterine adhesion	1.40 (0.85–2.16)	0.154	1.09 (0.65–1.72)	0.733		
Childbirth experience	0.61 (0.51–0.74)	<0.001	0.57 (0.42–0.75)	<0.001	0.55 (0.45–0.68)	<0.001
Number of abortions						
0	Ref					
1	1.12 (0.93–1.34)	0.214	1.00 (0.80–1.24)	0.964		
≥ 2	0.95 (0.72–1.23)	0.711	0.80 (0.58–1.09)	0.156		
Development of embryos	0.63 (0.27–1.25)	0.238	0.91 (0.37–1.93)	0.825		
Endometrial preparation	2.51 (2.14–2.95)	<0.001	2.19 (1.83–2.64)	<0.001	2.13 (1.79–2.52)	<0.001
EMT						
0.8 cm ≤ EMT ≤ 1.2 cm	Ref					
EMT < 0.8 cm	1.80 (1.37–2.33)	<0.001	1.73 (1.29–2.27)	<0.001	1.73 (1.31–2.27)	<0.001
EMT > 1.2 cm	1.15 (0.86–1.51)	0.337	1.40 (1.03–1.86)	0.025	1.39 (1.03–1.85)	0.028
Number of embryos	0.82 (0.59–1.11)	0.224	0.77 (0.54–1.07)	0.138	0.76 (0.54–1.04)	0.095
History of CS	0.67 (0.52–0.86)	0.002	0.90 (0.63–1.27)	0.538		

BMI Body mass index, *MAP* Mean arterial pressure, *GDM* Gestational diabetes mellitus, *EMT* Endometrial thickness, *PCOS* Polycystic ovary syndrome, *CS* cesarean delivery

an EMT between 0.8 cm and 1.2 cm. The group with the EMT of 0.8–1.2 cm was set as the reference group. A total of 8456 women delivered a singleton birth after FET with a natural cycle and 5002 women with an artificial protocol.



All basic characteristics in the three groups classified by EMT are shown in Table 1. There were significant differences were found in birth weight (3.38 ± 0.56 kg vs 3.46 ± 0.51 kg vs 3.45 ± 0.50 kg, $P < 0.001$), cesarean section rate (74.61% vs 69.15% vs 64.36%, $P < 0.001$), and HDP rate (7.89% vs 4.59% vs 5.24%, $P < 0.001$) among the three groups according to EMT. Mean EMT of the three groups were 0.72 ± 0.05 cm, 0.97 ± 0.12 cm, and 1.32 ± 0.08 cm, respectively.

Table 2 shows the univariate logistic regression analysis regarding related factors possibly associated with the risk of HDP. After adjusting the confounding factors, multivariate logistic regression still suggested that EMT was an independent risk factor of HDP. The increased risk of HDP after FET was associated with an EMT < 0.8 cm (aOR = 1.73; 95% CI, 1.31–2.27, $P < 0.001$) or > 1.2 cm (aOR = 1.39; 95% CI, 1.03–1.85, $P = 0.028$). Our study showed that maternal age (OR = 1.11; 95% CI, 1.02–1.21, $P = 0.022$), BMI (OR = 1.29; 95% CI, 1.20–1.39, $P < 0.001$), and GDM (OR = 1.79; 95% CI, 1.40–2.27, $P < 0.001$) were associated with a higher incidence of HDP after stepwise logistic regression analysis, whereas previous childbirth experience (OR = 0.55; 95% CI, 0.45–0.68, $P < 0.001$) was considered as a protective factor for HDP. For different endometrial preparations, the artificial

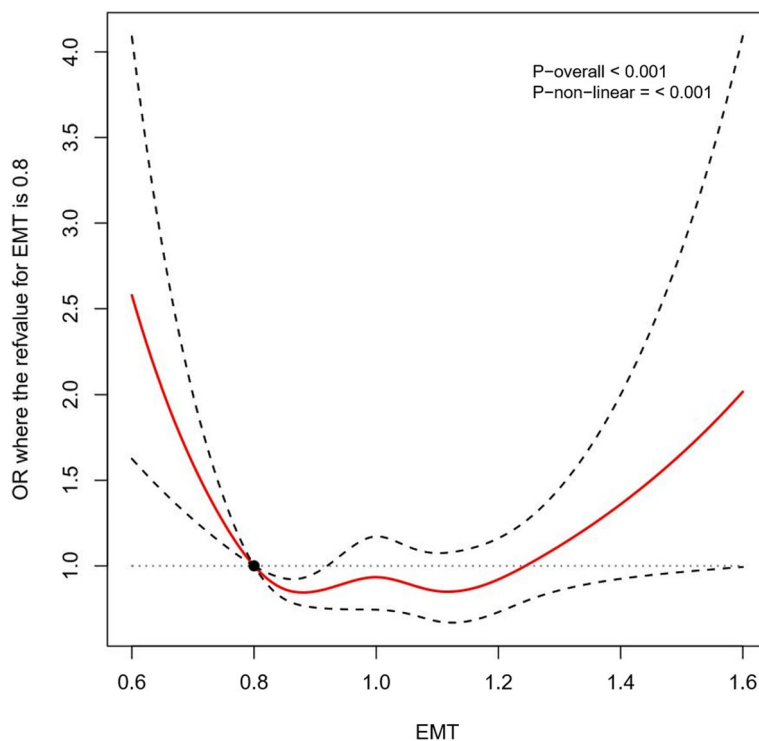


Fig. 2 The restricted cubic splines for EMT in association with HDP rate incorporating multivariate stepwise regression analysis model. (EMT = 0.8 cm as reference, knots = 5)

cycle was associated with a higher incidence of HDP (aOR = 2.13; 95% CI, 1.79–2.52, $P < 0.001$).

To evaluate the applicability of the current regression model, a ROC curve was created (Fig. 1). The area AUC reached 0.683, indicating that this model predicted the occurrence of HDP to a certain extent.

The results of restricted cubic spline (Fig. 2) demonstrated the non-linear relationship between EMT and the HDP rates ($P < 0.001$). With a reduction of EMT, the HDP risk significantly increased. The results showed that patients with an EMT ≤ 0.6 cm or an EMT around 0.7 cm ($0.6 \text{ cm} < \text{EMT} < 0.8 \text{ cm}$) were associated with an increased risk of HDP with ORs of 2.58 (95% CI, 1.63–4.09) and of 2.03 (95% CI, 1.44–2.86), respectively, compared with women with an EMT of 0.8 cm as a reference (Table 4). However, the dose relationship became insignificant with thicker EMT (1.3 cm, 1.4 cm, 1.5 cm, ≥ 1.6 cm) according to restricted cubic splines (Table 4). The probability of HDP in patients with an EMT of 0.8–1.2 cm reached the low ebb of the curve.

After subgroup stepwise logistic regression analysis based on endometrial preparation regimens (Table 3), the incidence of HDP increased significantly (aOR = 1.71; 95% CI, 1.22–2.33, $P = 0.001$) in patients with thin endometria prepared by an artificial cycle for FET, but

Table 3 Subgroup multivariate logistic regression analysis of EMT based on endometrial preparation regimen (Stepwise logistic regression)

Variable	Artificial cycle		Natural cycle	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Maternal age	1.01 (0.90–1.13)	0.843	1.23 (1.08–1.40)	0.002
Maternal BMI	1.23 (1.11–1.36)	< 0.001	1.39 (1.25–1.54)	< 0.001
Childbirth experience	0.64 (0.48–0.85)	0.002	0.46 (0.34–0.62)	< 0.001
EMT				
0.8 cm \leq EMT \leq 1.2 cm	Ref		Ref	
EMT < 0.8 cm	1.71 (1.22–2.33)	0.001	1.66 (0.94–2.74)	0.061
EMT > 1.2 cm	1.02 (0.54–1.78)	0.940	1.59 (1.11–2.21)	0.008
MAP	1.55 (1.38–1.74)	< 0.001	1.90 (1.66–2.18)	< 0.001
GDM	1.74 (1.25–2.38)	< 0.001	1.86 (1.28–2.65)	< 0.001
Number of embryos				
1	Ref		Ref	
> 1	0.72 (0.45–1.10)	0.151	0.81 (0.48–1.27)	0.382

EMT Endometrial thickness, MAP Mean arterial pressure, GDM Gestational diabetes mellitus

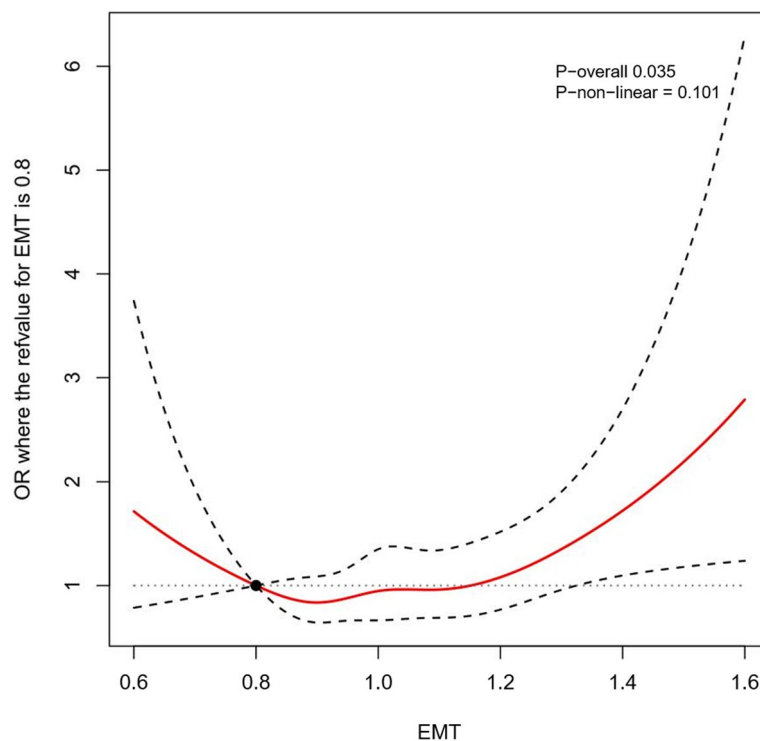


Fig. 3 The restricted cubic splines for EMT in association with HDP rate in natural cycle incorporating multivariate stepwise regression analysis model. (EMT=0.8 cm as reference, knots=5)

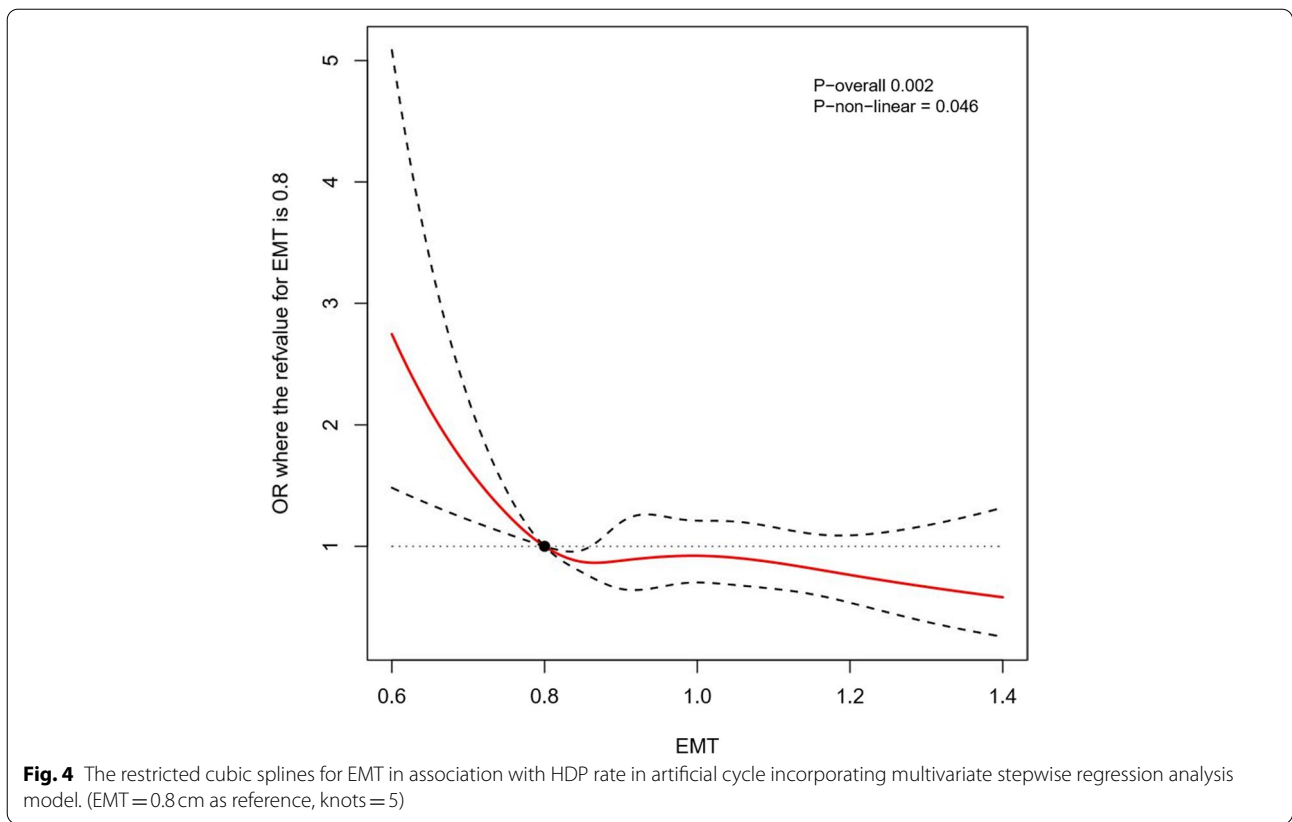
conversely, patients in the natural cycle group with thick endometria suffered more greatly from HDP than the reference group (aOR=1.59; 95% CI, 1.11–2.21, $P=0.008$). On the other hand, a thick EMT in artificial cycles did not increase the HDP risk and the odds ratio was not significant for HDP incidence in natural cycles with thin EMT after multivariate logistic regression analysis (aOR=1.02; 95% CI, 0.54–1.78, $P=0.940$; aOR=1.66; 95% CI, 0.94–2.74, $P=0.061$, respectively). These results were remeasured by the restricted cubic splines of natural cycles and artificial cycles separately (Figs. 3 and 4).

Discussion

HDP has received widespread attention owing to its negative impact on maternal and perinatal mortality. Hence, a retrospective cohort study of 13,458 single deliveries was performed to evaluate the association between EMT and HDP rate in FET cycles. We noted that both thin endometria and excessively thick endometria were identified as independent risk factors associated with HDP after adjusting for confounders. The non-linear dose-response relationship between EMT and HDP incidence in FET cycles was examined by restricted cubic spline.

Numerous studies have confirmed the risk of HDP with FET [10, 27], especially with programmed FET cycles, and the underlying mechanism is still unclear [19]. The main hypothesis is the absence of CL in artificial cycles that are commonly used for FET and this has been confirmed by both clinical and biological studies [28, 29]. The explanation is that CL produce not only estrogen and progesterone, but also vasoactive products including relaxin, vascular endothelial growth factor, and angiogenic metabolites of estrogen [30]. Therefore, programmed FET cycles are associated with a deficiency of these vasoactive products compared with FET cycles with CLs, including natural, modified natural, and stimulated cycles [29–31].

In addition to the absence of a CL, the clear etiology and pathophysiology of HDP is yet to be elucidated. It is now well known that placentation in humans is associated with unique vascular remodeling [32, 33]. Remodeling of the spiral arteries by extravillous trophoblasts (EVTs) is critical for adapting blood flow and nutrient transport to the developing fetus [34]. In pre-eclampsia, a major defect occurs in myometrial spiral artery remodeling [35–37]. The present literature suggests that the decidual environment critically modulates EVT function leading to reproductive



success [38]. Does a thin or a thick EMT influence EVT processes and induce superficial implantation? More research is needed to elucidate the correlation between EMT and EVT invasion, implantation, and placentation. In particular, a thin EMT might result from uterine curettage or infection, which could induce microcirculation and local immune changes, as

well as the inflammation. Recent studies have raised that these microenvironment changes would influence epigenetics progress in the placental formation by miRNAs expression [39–41]. Moreover, further large prospective studies on molecular biomarkers in excessive thin or deep endometrium, like soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor

Table 4 The multivariate stepwise regression analysis model incorporating restricted cubic splines for EMT in association with HDP rate

EMT (cm)	Overall		Natural cycle		Artificial cycle	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
0.6	2.42 (1.55–3.77)	2.58 (1.63–4.09)	1.69 (0.79–3.59)	1.71 (0.79–3.74)	2.38 (1.31–4.36)	2.75 (1.48–5.09)
0.7	1.93 (1.39–2.69)	2.03 (1.44–2.86)	1.48 (0.84–2.61)	1.50 (0.84–2.68)	1.89 (1.22–2.94)	2.10 (1.34–3.29)
0.8	Ref	Ref	Ref	Ref	Ref	Ref
0.9	0.86 (0.80–0.92)	0.86 (0.80–0.93)	0.88 (0.74–1.05)	0.88 (0.74–1.06)	0.89 (0.80–1.00)	0.87 (0.77–0.98)
1.0	0.85 (0.70–1.02)	0.90 (0.75–1.09)	0.89 (0.67–1.18)	0.88 (0.66–1.17)	1.00 (0.73–1.36)	0.91 (0.67–1.25)
1.1	0.78 (0.64–0.96)	0.89 (0.72–1.11)	1.01 (0.72–1.42)	0.96 (0.68–1.36)	1.05 (0.79–1.38)	0.90 (0.68–1.20)
1.2	0.72 (0.57–0.91)	0.86 (0.68–1.10)	1.09 (0.78–1.53)	1.00 (0.71–1.41)	0.97 (0.72–1.30)	0.82 (0.60–1.10)
1.3	0.81 (0.65–1.01)	1.02 (0.81–1.29)	1.31 (0.95–1.81)	1.21 (0.87–1.68)	0.85 (0.54–1.33)	0.71 (0.45–1.12)
1.4	0.95 (0.70–1.28)	1.24 (0.90–1.71)	1.62 (1.11–2.37)	1.54 (1.04–2.27)	0.75 (0.37–1.49)	0.62 (0.31–1.24)
1.5	1.10 (0.70–1.73)	1.51 (0.95–2.42)	2.01 (1.19–3.40)	1.96 (1.15–3.36)	–	–
1.6	1.28 (0.70–2.37)	1.84 (0.98–3.47)	2.50 (1.23–5.05)	2.50 (1.21–5.16)	–	–

EMT Endometrial thickness

(PIGF), natural killer (NK) cells, would help to clarify the pathophysiology of HDP [42–45].

The EMT is a priority in FET cycles and is relatively controllable. In the present study, the average EMT in artificial cycles was significantly thinner than in natural cycles. In an artificial cycle, once the EMT reached the basic criteria (around 0.8 cm), luteal support would usually be given rather than extending the proliferation phase to achieve a better thickness. However, in a natural cycle, the endometrium usually reaches an optimum thickness coincident with dominant follicle development and rupture. This might explain, why the endometrium was thicker in natural cycles than artificial cycles in clinical practice. The present study offers important evidence for positively adjusting EMT in FET cycles to improve perinatal outcome, especially in artificial cycles.

It is interesting that the influence of EMT on the incidence of HDP appeared in different patterns according to the two endometrial preparation regimens. The hypothesis is that the presence of CL might differently interact with endometria of varying thickness during decidualization, implantation, and EVT invasion. The etiology and physiology of this phenomenon urgently needs to be revealed. Nevertheless, the relatively small sample size of patients with a thick EMT in the artificial cycle group may have contributed to the result, to some extent. As shown in Table 1, in the thick EMT group, only 14.31% of patients were prepared by the artificial method.

Clinicians need to pay attention to those patients undergoing FET using an artificial method who have a thin EMT. Alternately, a natural cycle might be a better choice over an artificial protocol for patients with an inherently thin EMT. Furthermore, subgroup analysis results from the natural cycle group also raises questions as to whether women having a spontaneous pregnancy are influenced by EMT in the same way.

Another interesting question is whether differences in obstetrics and perinatal outcomes between fresh transfer and FET cycles, including HDP incidence, are partly induced by the difference in EMT. EMT might reach a relatively thicker state in controlled ovarian stimulation because of the extraordinary higher estradiol levels. Future studies are needed to illustrate this point.

To the best of our knowledge, this study is the first to identify the non-linear dose-response relationship between EMT and HDP risk in FET cycles. Unfortunately, in most published literature, EMT variability is commonly missing in analyses of association between the risk of HDP and FET treatment parameters. While in some other studies, EMT was taken as the covariates without stratification and failed to be estimated as a significant risk factor. Our study had three advantages compared to other studies. First,

the large sample size of this single-center study, in which clinical and IVF laboratory practices were uniformly controlled, reduced the potential bias and enhanced the statistical power. Second, we had strict inclusion and exclusion criteria. Finally, we adjusted a variety of potential predictors to minimize the potential effects of confounding factors. There were several limitations in our study. For the retrospective analysis, there were some intrinsic defects that could not be avoided. It is also important to note that some patients with a thin or thick endometrium proceeded with ET, while other patients canceled transfer. This might have created some bias and therefore may not reflected the true effect of thin or thick endometria. In addition, confounding factors known to influence pregnancy outcome, such as smoking and HDP history in previous pregnancies, were not recorded in the database.

Conclusion

The results of this study confirm that EMT in FET cycles is an independent risk factor on the development of pregnancy-induced hypertension. Clinicians should pay more attention to EMT during FET cycles for the benefit of maternal and child health. More research is needed to clarify the relationship between EMT and EVT invasion.

Abbreviations

HDP: Hypertensive disorders of pregnancy; EMT: Endometrial thickness; FET: Frozen-thawed embryo transfer; ART: Assisted reproductive technology; ET: Fresh embryo transfer; IVF/ICSI: In vitro fertilization/intracytoplasmic sperm injection; LGA: Large for gestational age; CL: Corpus luteum; ANOVA: One-way analysis of variance; OR: Odds ratio; 95%CI: 95% confidence intervals; aOR: Adjusted odds ratio; BMI: Body mass index; PCOS: Polycystic ovary syndrome; MAP: Mean arterial pressure; GDM: Gestational diabetes mellitus; OGTT: Oral glucose tolerance test; ROC curve: Receiver operating characteristic curve; AUC: Area under the curve; sFlt-1: Soluble fms-like tyrosine kinase-1; PIGF: Placental growth factor; NK cell: Natural killer cell.

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Authors' contributions

Shanshan Gao contributed to design of the study, writing-review and editing. Meng Zhang wrote the main manuscript text and analyzed the data. Jing Li, Xiao Fu and Yiting Zhang contributed to the review and the revision of the manuscript. Tao Zhang, Bingjie Wu and Xinyue Han contributed to statistical data analysis and prepared the figures. All authors reviewed and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Reproductive Medicine of Shandong University. This study does not involve human experiments, so the written informed consent of patients is not included.

Consent for publication

Not applicable.

Competing interests

We declare that we have no competing interests in present study.

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