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ARTICLE

Maternal and perinatal outcomes in programmed versus natural vitrified–warmed blastocyst transfer cycles

BIOGRAPHY

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KEY MESSAGE

Programmed frozen embryo transfer cycles are associated with more obstetric complications, particularly hypertensive disorders of pregnancy, compared with natural cycles. Therefore, natural frozen embryo transfer protocols should be recommended for endometrial preparation in eligible patients.

ABSTRACT

Research question: Do maternal and perinatal outcomes differ between natural and programmed frozen embryo transfer (FET) cycles?

Design: Retrospective cohort study at a university-affiliated fertility centre including 775 patients who underwent programmed or natural FET cycles resulting in a singleton live birth using blastocysts vitrified between 2013 and 2018.

Results: A total of 384 natural and 391 programmed FET singleton pregnancies were analysed. Programmed FET resulted in higher overall maternal complications (32.2% [126/391] versus 18.8% [72/384]; $P < 0.01$), including higher probability of hypertensive disorders of pregnancy (HDP) (15.3% [60/391] versus 6.3% [24/384]; $P < 0.01$), preterm premature rupture of membranes (2.6% [10/391] versus 0.3% [1/384]; $P = 0.02$) and caesarean delivery (53.2% [206/387] versus 42.8% [163/381]; $P = 0.03$) compared with natural FET. After controlling for potential confounders, including age, body mass index, parity, smoking status, history of diabetes or chronic hypertension, infertility diagnosis, number of embryos transferred and use of preimplantation genetic testing, the adjusted odds ratio for HDP was 2.39 (95% CI 1.37 to 4.17) and for overall maternal complications was 2.21 (95% CI 1.51 to 3.22) comparing programmed with natural FET groups. The groups did not significantly differ for any perinatal outcomes analysed, including birth weight (3357.9 ± 671.6 g versus 3318.4 ± 616.2 g; $P = 0.40$) or rate of birth defects (1.5% [6/391] versus 2.1% [8/384]; $P = 0.57$), respectively.

Conclusion: Vitrified–warmed blastocyst transfer in a programmed cycle resulted in a twofold higher probability of HDP compared with transfer in a natural cycle. Natural FET cycle should, therefore, be recommended as first line for all eligible patients undergoing FET to reduce the risk of HDP.

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KEYWORDS

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Obstetric outcome
Perinatal outcome
Vitrified–warmed embryo transfer

INTRODUCTION

Assisted reproductive technologies (ART) have been associated with an increased risk of obstetric and perinatal complications, including hypertensive disorders of pregnancy (HDP), antepartum haemorrhage, preterm delivery, low birthweight and congenital anomalies compared with spontaneous conception (*Helmerhorst et al., 2004*; *Jackson et al., 2004*; *Pandey et al., 2012*; *Hansen et al., 2013*; *Tandberg et al., 2015*; *Sunderam et al., 2019*). Hypertensive disorders of pregnancy refer to a spectrum of hypertensive disorders in pregnancy ranging from gestational hypertension to eclampsia. The higher incidence of multiple pregnancy after ART and proportion of women of advanced maternal age undergoing IVF may partly explain the increased occurrence of these complications (*Gardner et al., 1995*; *Tandberg et al., 2015*; *Qin et al., 2017*; *Sunderam et al., 2019*). Laboratory procedures, such as cryopreservation methods, extended blastocyst culture and use of preimplantation genetic testing (PGT), however, may also contribute to such adverse obstetric and perinatal outcomes (*Palomba et al., 2016*). In fact, compared with fresh embryo transfers, pregnancies after frozen embryo transfer (FET) cycles are at higher risk of HDP, placenta accreta, infants who are large for gestational age or have birth defects (*Roque et al., 2013*; *Wennerholm et al., 2013*; *Ishihara et al., 2014*; *Centers for Disease Control and Prevention, 2016*; *Maheshwari et al., 2016*; *Luke, 2017*; *Robles et al., 2017*). Given the growing utilization of FET cycles, which account for more than one-half of all the IVF cycles carried out in the USA, it is vital to identify the cause underlying the association between FET cycles and higher rates of such complications to improve patient safety and implement preventative measures.

Previous studies have suggested that particular aspects of FET cycle protocols may affect obstetric and perinatal outcomes. For example, a recent study demonstrated no difference in HDP between fresh transfer and natural FET cycles in ovulatory women (*Shi et al., 2018*) whereas the same research group previously showed a higher prevalence of HDP after programmed FET cycles compared with fresh IVF cycles in women with PCOS (*Chen et al., 2016*). This difference in results may be attributed to the method of endometrial preparation used by the disparate patient cohorts in the two studies. This is further supported by other studies that have shown that the risk of HDP does seem to be increased in programmed FET cycles compared with natural or modified natural cycles (*Ernstad et al., 2019*; *Saito et al., 2019*; *Versen-höynck et al., 2019a*). Additionally, the corpus luteum, which is present in natural and absent in programmed cycles, has been identified as possibly protective against the development of HDP (Arthur *et al*., 1996; Strauch *et al*., 2018; *Versen-höynck et al., 2019b*).

The nature of IVF treatment is rapidly changing, and it is challenging to obtain data that reflect new techniques and are generalizable to one's patient population. The two largest studies that have compared the difference in obstetric and perinatal outcomes between programmed and natural FET cycles were conducted internationally, limiting their generalizability to the US population (*Ernstad et al., 2019*; *Saito et al., 2019*). Furthermore, these studies included both cleavage- and blastocyst-stage embryo transfer cycles and one included both vitrification and slow freezing protocols for cryopreservation, although multicellular embryo transfer and slow freezing are no longer widely used (*Ernstad et al., 2019*; *Saito et al., 2019*). The only study using a US cohort included mainly blastocyst transfer and a few cleavage stage embryo transfer; however, it was limited by sample size and the cryopreservation technique used was unclear (*Versen-höynck et al, 2019a*; *2019b*).

Therefore, it is important to examine the effect that method of endometrial preparation has on obstetric and perinatal outcomes in a cohort of patients who underwent FET cycles using only previously vitrified blastocysts resulting in singleton birth to both reduce the effect of other cofounding variables and reflect current practice in US fertility centres. Therefore, we sought to determine whether maternal and perinatal outcomes differed between programmed and natural FET cycles involving transfer of previously vitrified blastocysts that resulted in a singleton live birth.

MATERIALS AND METHODS

Study design

This was a retrospective cohort study conducted at a single university-affiliated fertility centre comparing the difference between maternal and perinatal outcomes after programmed versus natural FET carried out between March 2013 and October 2018. Only cycles in which previously vitrified blastocyst(s) derived from autologous oocytes were transferred and resulted in a singleton live birth were included. Only one cycle per patient was included in the analyses. Exclusion criteria included donor oocyte cycles, FET cycles resulting in multiple births, cycles in which cleavage stage embryos were transferred or cycles in which the embryos that were transferred had been cryopreserved using slow freezing. In addition, modified natural FET cycles involving ovulation induction with letrozole, clomiphene citrate or gonadotrophins, as well as those requiring HCG triggering to induce ovulation, were excluded. This study was approved by our university's institutional review board on 15 February 2019.

Treatment protocol

IVF stimulation protocols have been previously described and were decided based on patient factors and physician preference (*Diluigi et al., 2011*; *Johnston-MacAnanny, et al., 2011*). Gonadotrophin dosing was adjusted according to patient response. Trigger for final oocyte maturation was administered when three or more follicles reached 18 mm in diameter or above. Either HCG 3300–10000 IU SC (Pregnyl, Merck, Kenilworth, NJ, USA; Novarel, Ferring Pharmaceuticals, Parsippany, NJ, USA), gonadotrophin releasing hormone (GnRH) agonist 1 mg (leuprolide acetate, Abbott Laboratories, Abbott Park, IL, USA) or a combination was used for trigger based on physician preference, protocol type and risk of ovarian hyperstimulation syndrome. Transvaginal ultrasound-guided oocyte retrieval was carried out 35 h after trigger injection. Oocytes were fertilized with either intracytoplasmic sperm injection or conventional insemination.

Only good-quality blastocysts according to Gardner criteria (3BB or higher) (*Gardner and Schoolcraft, 1999*) were vitrified. If PGT was being used, trophectoderm biopsy was carried out, embryos vitrified and either array

comparative genomic hybridization or next-generation sequencing was used for aneuploidy testing. Vitrification was by rapid exposure to a cryoprotectant solution consisting of 15% ethylene glycol, 15% dimethylsulphoxide, 20% dextran and 0.5 mmol/l sucrose in a Cryolock® device (Irvine Scientific, Santa Ana, CA, USA). Before vitrification, a laser pulse of 300 µs (constant 0.9 J) was applied to collapse the blastocoel. Before embryo transfer, all blastocysts were rapidly warmed in solutions of 0.5 and 0.2 mmol/l sucrose and rinsed through HEPES-buffered human tubal fluid with 12 mg/ml human serum albumin. To allow time for re-expansion, embryos were warmed and equilibrated in culture 1–2 h before transfer.

As previously described (*Kaye et al., 2018*), FET was carried out in either a natural or programmed cycle. The decision to use a programmed or natural FET cycle was based on the patient's ovulatory status and physician preference. Programmed cycles consisted of downregulation with a GnRH agonist in the luteal phase of the preceding cycle followed by increasing doses of oral or transdermal oestradiol after menses. Intramuscular progesterone was started when endometrial thickness measured about 8 mm. On the sixth day of intramuscular progesterone, FET was carried out. In a natural FET cycle, patients were monitored with daily bloodwork beginning on cycle day 10. A transvaginal ultrasound was also carried out at this time to ensure a follicle had been recruited and that the endometrial stripe measured about 8 mm. Six days after detecting the LH surge (LH ≥20 IU/l), FET was carried out and the luteal phase supplemented with vaginal progesterone (Crinone, Merck, Kenilworth, NJ, USA; and Endometrin, Ferring Pharmaceuticals, Parsippany, NJ, USA), commencing 2 days after the LH surge (*Bartels et al., 2019*).

Outcome measures

Demographic and baseline cycle information were collected from the electronic medical records at our fertility centre, including age, body mass index (BMI), smoking status, parity, history of pre-existing diabetes or chronic hypertension (HTN), primary infertility diagnosis, mean number of embryos transferred and use of PGT. Outcome data, including mode of delivery and occurrence of maternal or perinatal complications, was self-reported and sent to the Society of Assisted Reproductive Technology. These data were collected by nurses who telephoned each patient after delivery to obtain delivery details and information on any complications.

The primary outcomes were overall rate of maternal complications, particularly HDP, which included the diagnoses of pre-eclampsia, gestational hypertension, the syndrome of haemolysis, elevated liver enzymes and low platelet count or eclampsia. Secondary outcomes included maternal delivery characteristics and rate of other obstetric complications, e.g. mode of delivery, gestational diabetes, postpartum haemorrhage, preterm premature rupture of membranes (PPROM), chorioamnionitis, placenta accreta, placenta previa, postpartum haemorrhage and placental abruption, as well as perinatal outcomes (rate of birth defects, birth weight, gestational age at birth, newborn intensive care unit admission and fetal loss.) Birth defects were classified as major and minor as described in previous studies (*Bonduelle et al., 2002*). A major congenital anomaly was defined as a condition that reduces the viability or compromises the quality of life and requires medical treatment or a condition that causes functional impairment or requires surgical correction.

Statistical analysis

IBM SPSS© Statistics version 26.0 was used for statistical analysis. Student's t-test or Mann–Whitney U was used to analyse continuous variables depending on whether the variable was normally distributed. Chi-squared or Fischer's exact test was used for categorical variables and, where indicated, a post-hoc analysis involving pairwise comparisons was conducted if there were three or more independent groups, using z-test of two proportions with a Bonferroni correction. Continuous variables were presented as mean ± SD, and categorical variables presented as percentage and count. A binary logistic regression was conducted to control for potential covariates that may be associated with an increased risk of HDP, including age, BMI, and number of embryos transferred as continuous variables and smoking status, parity, history of diabetes mellitus or HTN, primary infertility diagnosis and use of PGT as categorical variables (*Vidaeff et al., 2019*; *Zhang et al., 2019*). All the variables were entered using a forced entry method, and all the predictor variables were tested in one block to assess their predictive ability while controlling for other predictors in the model. Both crude and adjusted odds ratios with 95% confidence intervals were calculated. In addition, subgroup analyses were conducted stratifying groups by age (<35 and ≥35 years), BMI (≤30 and >30 kg/m^2) and use of PGT. A two-sided *P*-value of less than 0.05 was considered statistically significant. Bonferroni correction, however, was used for multiple comparisons. For the 10 maternal outcomes assessed, $P < 0.005$ would be considered statistically significant. For the 15 perinatal outcomes assessed, $P < 0.003$ would be considered statistically significant.

RESULTS

Baseline characteristics

The baseline cycle and demographic information for each FET group of patients is presented in TABLE 1. A total of 775 FET cycles comprising 384 natural and 391 programmed FET cycles were included for analysis. Women who underwent natural FET cycles were significantly older (35.0 ± 3.7 versus 33.9 ± 3.9 years; $P < 0.01$) and had lower BMI (25.9 ± 5.7 versus 27.1 ± 6.2kg/m^2; $P < 0.01$) than those who underwent programmed FET, respectively. Women who underwent natural FET cycles were also more likely to have used PGT ($P = 0.01$) and transferred fewer embryos ($P < 0.01$). Infertility diagnosis was significantly different between the two groups (TABLE 1). To determine which groups were significantly different, a post-hoc analysis was conducted involving pairwise comparisons using the z-test of two proportions with a Bonferonni correction. This showed that a significantly higher proportion of women with the diagnosis of anovulation or polycystic ovary syndrome used a programmed FET protocol compared with a natural cycle. In contrast, more women with unexplained or male factor infertility underwent a natural FET protocol. A comparable number of women in both groups were smokers and primigravida at time of conception. No differences were observed between groups in the proportion of women with history of diabetes mellitus and HTN.

Maternal outcomes

Maternal delivery characteristics and outcomes are presented in TABLE 2

TABLE 1 BASELINE CHARACTERISTICS FOR NATURAL AND PROGRAMMED FROZEN EMBRYO TRANSFER GROUPS

Baseline characteristics	*Natural FET (n = 384)*	*Programmed FET (n = 391)*	*P-value*
Age, years, mean ± SD	35.0 ± 3.7	33.9 ± 3.9	<0.01
Age group, % (*n*)			<0.01
<35 years	49.7 (191/384)	62.9 (246/391)	
≥35 years	50.3 (193/384)	37.1 (145/391)	
BMI (kg/m^2), mean ± SD)	25.9 ± 5.7	27.1 ± 6.2	<0.01
BMI group, % (*n*)			0.03
≤30 kg/m^2	79.2 (304/384)	72.6 (284/391)	
>30 kg/m^2	20.8 (80/384)	27.4 (107/391)	
Smoker, % (*n*)	3.1 (12/384)	3.3 (13/389)[a]	0.87
Parity, % (*n*)			0.43
Parous	32.6 (125/384)	29.9 (117/391)	
Nulliparous	67.4 (259/384)	70.1 (274/391)	
History of pre-existing diabetes, % (*n*)			0.25
Yes	0.0 (0/384)	0.8 (3/391)	
No	100.0 (384/384)	99.2 (388/391)	
History of chronic HTN, % (*n*)			0.22
Yes	0.3 (1/384)	1.3 (5/391)	
No	99.7 (383/384)	98.7 (386/391)	
Diagnosis, % (*n*)			<0.01
Unexplained	26.6 (102/384)	12.0 (47/391)	
Anovulation/PCOS	3.9 (15/384)	37.3 (146/391)	
Male factor	31.0 (119/384)	21.2 (83/391)	
DOR	4.7 (18/384)	2.8 (11/391)	
Endometriosis	6.5 (25/384)	7.2 (28/391)	
Fibroids	3.9 (15/384)	2.3 (9/391)	
RPL	8.9 (34/384)	7.2 (28/391)	
Tubal factor	8.3 (32/384)	6.1 (24/391)	
Other	6.3 (24/384)	3.8 (15/391)	
Cycle characteristics			
Mean number of embryos transferred (*n*, mean ± SD)	1.2 ± 0.4	1.3 ± 0.5	<0.01
PGT, % (*n*)	35.2 (135/384)	26.3 (103/391)	0.01

[a] The denominator is less than total number of patients in the group because of missing data for that variable.

BMI, body mass index; DOR, diminished ovarian reserve; HTN, chronic hypertension; PCOS, polycystic ovary syndrome; PGT, preimplantation genetic testing; RPL, recurrent pregnancy loss.

and observations specific to HDP are shown in FIGURE 1. Programmed FET was associated with more overall maternal complications (32.2% [126/391] versus 18.8% [72/384]; $P < 0.001$) than natural FET, respectively. More specifically, women in the programmed FET group had a higher probability of HDP than the natural FET group (15.3% [60/391] versus 6.3% [24/384]); $P = 0.001$), respectively. Moreover, women in the programmed FET group more often experienced PPROM and were delivered via caesarean section. Only the rate of overall maternal complications and rate of HDP, however, remained significantly different after the Bonferroni correction was applied. No significant difference was observed between groups in the rate of gestational diabetes mellitus or placenta accreta, although a few cases of placenta accreta were reported.

In view of the differences in baseline characteristics as well as known risk factors for HDP, subgroup analyses were conducted. These subgroup analyses showed that a higher probability of HDP in the programmed compared with natural FET group generally persisted even after groups were stratified by age (15.9% [39/246] versus 6.8% [13/191], $P < 0.01$; 14.5% [21/145] versus 5.7% [11/193], $P < 0.01$, $P < 35$ and $P \geq 35$ years, respectively) (FIGURE 1A), BMI (12.0% [34/284] versus 3.9% [12/304], $P < 0.01$; 24.3% [26/107] versus 15.0% [12/80], $P = 0.12$), BMI ≤30 and >30 kg/m^2, respectively) (FIGURE 1B) and the use of PGT (13.9% [40/288] versus 5.2% [13/249], $P < 0.01$; 19.4% [20/103] versus 8.1% [11/135], $P = 0.01$, without PGT versus with PGT, respectively) (FIGURE 1C). Although HDP was significantly higher in programmed compared with natural

TABLE 2 MATERNAL OUTCOMES FOR NATURAL AND PROGRAMMED FROZEN EMBRYO TRANSFER GROUPS

Maternal complications	*Natural FET*	*Programmed FET*	*Crude OR (95% CI)*	*Adjusted OR (95% CI)*[a]	P- *value*[a]
Overall, %, *n*	18.8 (72/384)	32.2 (126/391)	2.06 (95% CI 1.48 to 2.87)	2.21 (95% CI 1.51 to 3.22)	<0.01
Gestational diabetes, %, *n*	7.3 (28/384)	10.7 (42/391)	1.53 (95% CI 0.93 to 2.52)	1.69 (95% CI 0.95 to 2.99)	0.07
Hypertensive disorders of pregnancy, %, *n*	6.3 (24/384)	15.3 (60/391)	2.56 (95% CI 1.56 to 4.25)	2.39 (95% CI 1.37 to 4.17)	<0.01
Preterm premature rupture of membranes, %, *n*	0.3 (1/384)	2.6 (10/391)	10.05 (95% CI 1.28 to 78.91)	11.71 (95% CI 1.44 to 95.55)	0.02
Chorioamnionitis, % (*n*)	0.5 (2/384)	0.5 (2/391)	0.98 (95% CI 0.25 to 8.89)	0.76 (95% CI 0.08 to 7.17)	0.81
Placental abruption, % (*n*)	0.5 (2/384)	0.8 (3/391)	1.48 (95% CI 0.25 to 8.94)	1.11 (95% CI 0.12 to 10.15)	0.93
Placenta accrete, % (*n*)	0.3 (1/384)	0.5 (2/391)	1.97 (95% CI 0.18 to 21.81)	2.98 (95% CI 0.25 to 34.95)	0.38
Placenta previa, (%) *n*	2.3 (9/384)	2.6 (10/391)	1.09 (95% CI 0.44 to 2.72)	1.50 (95% CI 0.57 to 3.89)	0.42
Postpartum haemorrhage, % (*n*)	1.0 (4/384)	1.3 (5/391)	1.23 (95% CI 0.33 to 4.62)	2.02 (95% CI 0.49 to 8.30)	0.33
Caesarean delivery, % (*n*)	42.8 (163/381)[b]	53.2 (206/387)[b]	1.52 (95% CI 1.14 to 2.02)	1.44 (1.04 to 2.01)	0.03

[a] Binary logistic regression controlling for covariates, including age, body mass index, smoking status, parity, history of diabetes mellitus or chronic hypertension, primary infertility diagnosis, number of embryos transferred and use of preimplantation genetic testing.

[b] The denominator is less than total number of patients in the group because of missing data for that outcome variable.

FET, frozen embryo transfer.

FET cycles in women with BMI 30 kg/m^2 or above, statistical significance was not reached for HDP rates in programmed FET cycles in women with BMI above 30 kg/m^2, mainly because of the relatively smaller sample size in the BMI above 30 kg/m^2 group. After adjusting for covariates of age, BMI, diagnosis, smoking status, history of diabetes mellitus or HTN, primary infertility diagnosis, number of embryos transferred and use of PGT, the probability of developing HDP was twofold higher in women who used programmed compared with natural FET cycles (adjusted OR 2.39; 95% CI 1.37 to 4.17; $P < 0.01$) (TABLE 2).

In addition, a secondary analysis was conducted in which patients with anovulatory infertility were excluded because they may have a higher likelihood of maternal complications, which could confound results. When patients with anovulatory infertility were excluded from the analysis, the rate of HDP remained significantly higher in the programmed FET group compared with the natural FET group (15.1% [37/245] versus 6.5% [24/369]; $P = 0.001$), respectively. Similarly, the rate of overall maternal complications also remained significantly higher in the programmed FET group (34.7% [85/245] versus 19.0% [70/369]; $P < 0.001$) compared with the natural FET group, respectively. In this subgroup analysis, the odds ratio for HDP was 2.56 (95% CI 1.49 to 4.40) and for overall maternal complications was 2.27 (95% CI 1.57 to 3.29).

Perinatal outcomes

No significant difference was observed between groups in any of the perinatal outcomes, including the mean birth weight and the probability of having an infant with birth defects (TABLE 3). Furthermore, no statistically significant difference was found in mean birthweight between groups, including the proportion of macrosomic infants (≥4000 g) in the programmed compared with the natural FET groups (14.0% [54/386] versus (9.4% [36/381]; $P = 0.07$)). In addition, no minor birth defects were reported in either group and no significant difference was found between groups in reported major birth defects (2.1% [8/384] versus 1.5% [6/391]; $P = 0.57$).

DISCUSSION

Our study evaluated the effect of endometrial preparation on obstetric and perinatal outcomes in a large US cohort, which included only singleton deliveries after transfer of blastocysts that were previously vitrified. We showed that the probability of developing HDP was twofold higher in women who conceived after programmed compared with natural FET cycles (adjusted OR 2.39, 95% CI 1.37 to 4.17; $P < 0.01$). This result persisted after controlling for important covariates known to be risk factors for developing HDP, including age, BMI, diagnosis, smoking status, history of diabetes mellitus or HTN, primary infertility diagnosis, number of embryos transferred and use of PGT. We did not detect any differences in perinatal outcomes between the two methods of endometrial preparation.

These results have significant implications for current practice patterns in US fertility centres. Previous studies comparing methods for endometrial preparation in FET protocols demonstrated equivalent pregnancy outcomes, including clinical pregnancy, ongoing pregnancy and live birth rates (*Groenewoud et al., 2013*; *Peeraer et al., 2015*; *Yarali et al., 2016*). Given this information, decisions about which protocol to choose for FET cycles have largely been left to physician preference if a particular patient's characteristics allowed for both protocol types. In fact, programmed FET is often selected simply because it offers the most flexibility in terms of scheduling owing to the ability to avoid weekend embryo transfers. Our results argue against this approach and provide further justification to strongly recommend natural over programmed FET cycles in all eligible patients.

Our results are consistent with the findings of previous studies, which most notably demonstrate a significantly higher risk of HDP in pregnancies conceived after programmed FET cycles compared with natural FET cycles (*Ernstad et al., 2019*; *Saito et al., 2019*; *Versen-höynck et al., 2019b*). In the present study, this held true even after stratification and controlling for covariates that are potential risk factors for the development of HDP. Our results, more importantly, confirm an increased risk

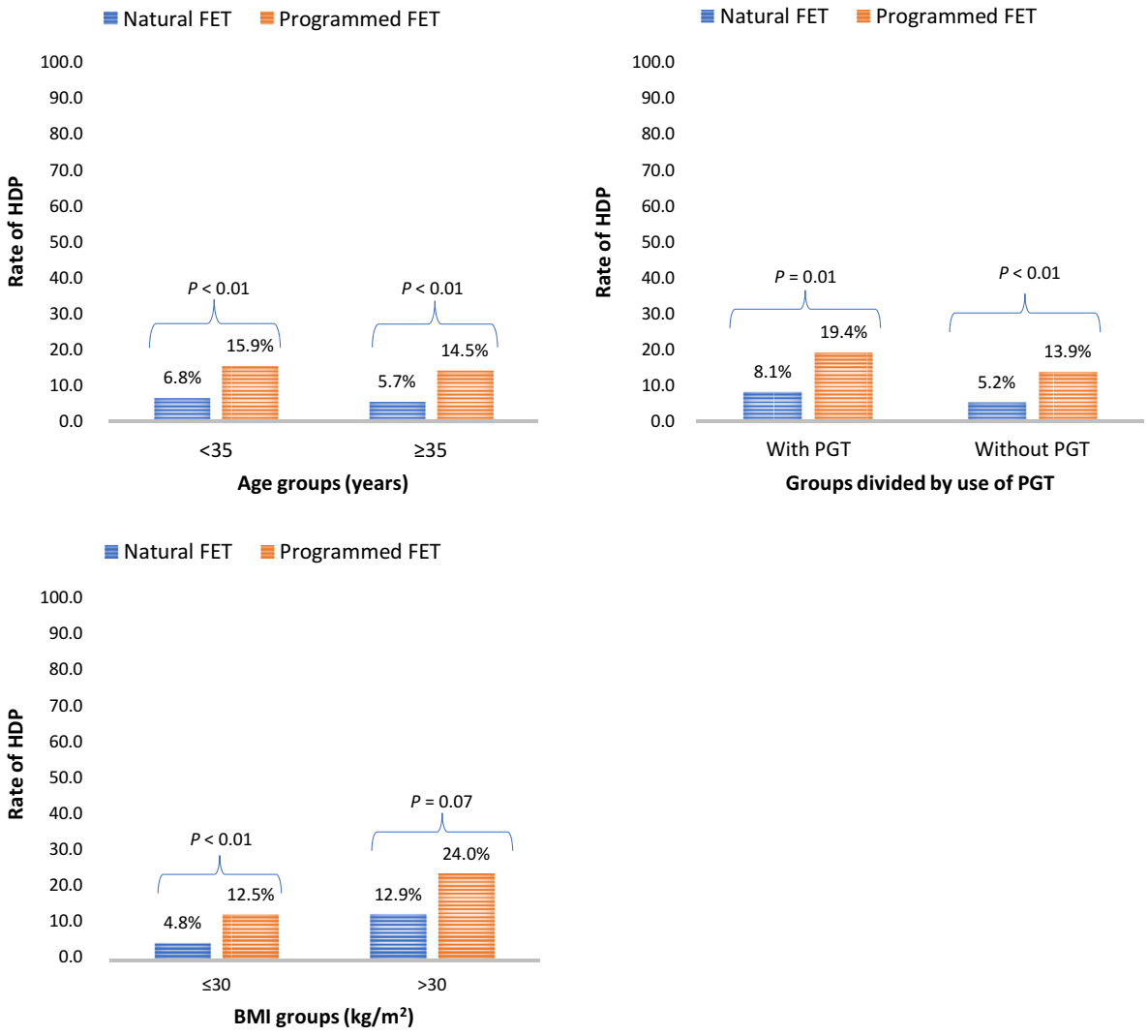

FIGURE 1 Rate of hypertensive disorders in pregnancy stratified by (A) age; (B) body mass index; and (C) use of preimplantation genetic testing for aneuploidies. BMI, body mass index; FET, frozen embryo transfer; HDP, hypertensive disorders of pregnancy; PGT, preimplantation genetic testing.

of HDP in current IVF practices, which include blastocyst culture, vitrification cryopreservation protocol and use of modern PGT techniques.

To the best of our knowledge, this is the largest US cohort of patients that addresses differences in maternal and perinatal outcomes after programmed and natural FET cycles. Two previous studies with large cohorts addressing this question have been conducted internationally, limiting their generalizability to our population (*Ernstad et al., 2019*; *Saito et al., 2019*). Both of these studies, one conducted in a Swedish cohort and the other in a Japanese cohort, demonstrated a higher rate of HDP in programmed FET cycles compared with natural FET cycles. They both, however, included a significant number of cycles with transfer of cleavage-stage embryos (>50% of the embryos in the Swedish study and 20–30% of the embryos in the Japanese study.) Furthermore, in the Swedish study, embryos were transferred that had been cryopreserved using slow freezing. Although the investigators state that they controlled for freezing method, they did not specify what proportion of cycles used slow freezing. Nevertheless, given that their registry included cycles spanning back as far as 2005, one can assume that a significant number of cycles were carried out before the widespread adoption of vitrification. One prospective study has been conducted in a US cohort. The investigators found a significantly higher rate of HDP in programmed FET cycles. Although their analysis covered only procedures carried out in recent years (2011–2017) and mostly involved blastocyst transfers (>95%), their results were limited by sample size, and it was unclear what proportion of embryos were cryopreserved using slow freezing. Furthermore, it was also unclear whether multiple cycles per patient were included, which would affect their results (*Versen-höynck et al., 2019b*).

It has been proposed that this heightened risk of HDP is because programmed FET protocols do not mimic physiologic conditions, resulting in abnormal placentation (*Conrad et al., 2017*). It is accepted that HDP disorders develop from uteroplacental insufficiency (*Mol et al., 2019*); however, more recently it has been proposed that imbalances in angiogenic factors play a role in the pathogenesis of HDP (*Vidaeff et al., 2019*). *Versen-Höynck et al. (2019a*; *2019b*) were the first to show that the

TABLE 3 PERINATAL OUTCOMES FOR NATURAL AND PROGRAMMED FROZEN EMBRYO TRANSFER GROUPS

Perinatal birth characteristics and complications	*Natural FET*	*Programmed FET*	*P-value*
Mean gestational age, weeks, mean ± SD	38.7 ± 2.3	38.7 ± 3.2	0.85
Gestational age group, % (*n*)			0.59
≤28 weeks	1.0 (4/384)	1.5 (6/391)	
>28 to <34 weeks	1.8 (7/384)	3.1 (12/391)	
>34 to <37 weeks	9.6 (37/384)	8.4 (33/391)	
≥37 weeks	87.5 (336/384)	87.0 (340/391)	
Weight, g, mean ± SD	3318.4 ± 616.2	3357.9 ± 671.6	0.40
Weight group, % (*n*)			0.14
≤2500 g	7.1 (27/381)[a]	8.0 (31/386)[a]	
>2500 to <4000 g	83.5 (318/381)	78.0 (301/386)	
≥4000 g	9.4% (36/381)	14.0 (54/386)	
Overall complications, % (*n*)	16.1 (62/384)	18.7 (73/391)	0.35
NICU admission, % (*n*)	4.7 (18/394)	6.4 (25/391)	0.30
Fetal loss <24 weeks, % (*n*)	0.3 (1/384)	0.3 (1/391)	1.0
Fetal loss ≥24 weeks, % (*n*)	0.0 (0/384)	0.0 (0/391)	1.0
Major birth defects, % (*n*)	2.1 (8/384)	1.5 (6/391)	0.57
Minor birth defects, % (*n*)	0.0 (0/384)	0.0 (0/391)	1.0

[a] The denominator is less than total number of patients in the group because of missing data for that outcome variable.

FET, frozen embryo transfer; NICU, newborn intensive care unit.

absence of corpus luteum may affect vascular health during early pregnancy by altering maternal circulation. In an initial study, the investigators analysed a small cohort of patients undergoing IVF based on number of corpus luteum present, and found that, in the absence of a corpus luteum, mean arterial pressure did not decline as expected in pregnancy and also that the measures of endothelial cell function and arterial stiffness were significantly altered. In addition, circulating angiogenic progenitor cells were lower and the concentration of relaxin, a potent vasodilator secreted by corpus luteum was lower; this contributes to maternal vascular adaptations in pregnancy as shown in animal models (*Marshall et al., 2016*). In a subsequent study, they further showed that vascular compliance was blunted in the absence of a corpus luteum (*Arthur et al., 1996*; *Strauch et al., 2018*; *Versen-höynck et al., 2019b*; *2019a*). On the basis of these findings, one might argue that natural or modified natural FET protocols, in which one or more corpora lutea are present, should be recommended as the FET protocols of choice for eligible patients to reduce their risk of HDP.

We observed a higher rate of PPROM in the programmed compared with the natural FET group. When the Bonferroni correction was applied, however, this result was no longer significant. Furthermore, there does not seem to be a physiological explanation for the increased rate of PPROM. Moreover, we did not find an increased risk of preterm delivery, so it is unlikely that this finding is clinically significant. Additionally, unlike previous reports (*Ishihara et al., 2014*; *Saito et al., 2019*), we did not find a higher rate of placenta accreta in programmed FET pregnancies. It is reasonable to assume that, if placental development is compromised in programmed FET cycles, there should be a higher risk of disorders of abnormal placentation, including placenta accreta and placenta previa. Placenta accreta, however, is a rare event, ranging from 1 in 272 to 1 in 533 pregnancies (*American College of Obstetricians and Gynecologists and Society for the Maternal-Fetal Medicine, 2018*). It is, therefore, notable that, in the study by *Saito et al. (2019)*, the prevalence of placenta accreta was less than 1% in both the programmed and natural cycles groups (0.1% versus 0.9%; $P < 0.01$), which is consistent with the probability noted in our study (0.3% versus 0.5%, $P = 1.0$). They found a statistically significant difference, however, because of their large sample size and the clinical significance of this finding is debatable. Similarly, we did not detect a significant difference in gestational diabetes mellitus as described by *Saito et al. (2019)*.

Although multiple studies have demonstrated a higher incidence of macrosomic infants born after programmed FET (*Ishii et al., 2018*; *Ernstad et al., 2019*; *Saito et al., 2019*), we did not detect a significant difference in mean birth weight according to FET protocol nor when birth weight was stratified into groups. In general, it is difficult to reconcile how programmed FET cycles result in both high rates of HDP, which is often associated with fetal growth restriction, and fetal macrosomia. Potential explanations may include distinctive parental traits, such as more gestational diabetes mellitus in women who give birth to macrosomic infants. We also did not detect a significant difference in major or minor birth defects, but as birth defects are so rare, a much larger data set may be required to discern such a difference.

Our study is unique because we included only singleton deliveries that resulted from transfer of vitrified–warmed autologous blastocysts. One may argue that the findings of this study are limited by the fact that all cycles were performed at a single fertility centre, but it is also a strength as each group was treated uniformly in the medications that were

used and how they were monitored. In contrast, in the Swedish cohort study of 10,000 singleton births discussed above (*Ernstad et al., 2019*), the type of FET cycle carried out and medications used were assumed based on what drugs had been prescribed for each patient. Therefore, it was possible that centres across the country were using vastly different protocols and that the medications prescribed were not the ones necessarily used by the patient. Moreover, our study was conducted retrospectively, so there is a risk of confounding and bias. Logistic regression was conducted to control for potential significant confounders, including age, BMI, smoking status, parity, history of pre-existing diabetes or HTN, primary infertility diagnosis, number of embryos transferred and use of PGT, and still found a significantly higher risk of HDP in the programmed FET group. Furthermore, as significantly more women had anovulatory infertility in the programmed FET group, we carried out a secondary analysis and excluded these patients as they may have a higher probability of maternal complications. We still found that rate of HDP and overall maternal complications was significantly higher in the programmed FET group.

Nevertheless, we recognize that other possible confounders may exist that that we could not account for, such as history of other concurrent medical conditions that may increase the risk of HDP (renal disease, autoimmune disease) or prior history of HDP. In addition, we did not have information on vanishing twins or fetal demise of a twin. Research suggests that pregnancies complicated by vanishing twin syndrome may be associated with increased perinatal complications, including low birthweight and preterm delivery (*Almog et al., 2010*; *Evron et al., 2015*; *Timur et al., 2018*). To our knowledge, however, vanishing twin syndrome has not been associated with increased risk of HDP. Nevertheless, it may represent a potential confounder for outcomes. As such, we hope that our results prompt further investigation. A prospective randomized controlled trial would be the optimal method to validate our findings.

In conclusion, blastocyst transfer in a programmed FET cycles is associated with a higher risk of overall maternal complications, particularly HDP. It is important to highlight that HDP is one of the leading causes of maternal and perinatal death around the world (*Vidaeff et al., 2019*). Currently, no accurate screening methods are available for the early detection of women at risk of developing HDP. Low-dose aspirin may modestly reduce the risk of developing HDP, but no interventions can completely eliminate it (*ACOG, 2018*). Therefore, the most effective strategy to reduce a patient's risk for HDP may be to evaluate for modifiable risk factors, one of which is selecting the type of FET protocol type used by women during IVF. As such, natural FET protocols should be recommended as first-line for all eligible patients undergoing IVF to reduce the risk of HDP.

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