Human Reproduction, Vol.34, No.8, pp. 1567–1575, 2019
Advance Access Publication on July 12, 2019 doi:10.1093/humrep/dez079

human reproduction

ORIGINAL ARTICLE ***Reproductive epidemiology***

Endometrial preparation methods for frozen-thawed embryo transfer are associated with altered risks of hypertensive disorders of pregnancy, placenta accreta, and gestational diabetes mellitus

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Submitted on February 4, 2018; resubmitted on April 24, 2019; editorial decision on April 29, 2019

STUDY QUESTION: What were the risks with regard to the pregnancy outcomes of patients who conceived by frozen-thawed embryo transfer (FET) during a hormone replacement cycle (HRC-FET)?

SUMMARY ANSWER: The patients who conceived by HRC-FET had increased risks of hypertensive disorders of pregnancy (HDP) and placenta accreta and a reduced risk of gestational diabetes mellitus (GDM) in comparison to those who conceived by FET during a natural ovulatory cycle (NC-FET).

WHAT IS KNOWN ALREADY: Previous studies have shown that pregnancy and live-birth rates after HRC-FET and NC-FET are comparable. Little has been clarified regarding the association between endometrium preparation and other pregnancy outcomes.

STUDY DESIGN, SIZE, DURATION: A retrospective cohort study of patients who conceived after HRC-FET and those who conceived after NC-FET was performed based on the Japanese assisted reproductive technology registry in 2014.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The pregnancy outcomes were compared between NC-FET (n = 29 760) and HRC-FET (n = 75 474) cycles. Multiple logistic regression analyses were performed to investigate the potential confounding factors.

MAIN RESULTS AND THE ROLE OF CHANCE: The pregnancy rate (32.1% vs 36.1%) and the live birth rate among pregnancies (67.1% vs 71.9%) in HRC-FET cycles were significantly lower than those in NC-FET cycles. A multiple logistic regression analysis showed that pregnancies after HRC-FET had increased odds of HDPs [adjusted odds ratio, 1.43; 95% confidence interval (CI), 1.14–1.80] and placenta accreta (adjusted odds ratio, 6.91; 95% CI, 2.87–16.66) and decreased odds for GDM (adjusted odds ratio, 0.52; 95% CI, 0.40–0.68) in comparison to pregnancies after NC-FET.

LIMITATIONS, REASONS FOR CAUTION: Our study was retrospective in nature, and some cases were excluded due to missing data. The implication of bias and residual confounding factors such as body mass index, alcohol consumption, and smoking habits should be considered in other observational studies.

WIDER IMPLICATIONS OF THE FINDINGS: Pregnancies following HRC-FET are associated with higher risks of HDPs and placenta accreta and a lower risk of GDM. The association between the endometrium preparation method and obstetrical complication merits further attention.

STUDY FUNDING/COMPETING INTEREST(S): No funding was obtained for this work. The authors declare no conflicts of interest in association with the present study.

TRIAL REGISTRATION NUMBER: Not applicable.

Key words: frozen-thawed embryo transfer / gestational diabetes mellitus / hormone replacement cycle / hypertensive disorders of pregnancy / placenta accreta

Introduction

Frozen-thawed embryo transfer (FET) enables the storage of excess embryos produced through in-vitro fertilization or intracytoplasmic sperm injection. FET reduces the waste of embryos and repeated oocyte retrieval, allowing patients to attempt embryo transfer several times if multiple embryos are obtained from a single oocyte retrieval. In addition, improvements in the pregnancy rate after FET encourage physicians to transfer fewer embryos (Takeshima *et al.*, 2016). Thus, the rates of multiple pregnancies and obstetrical complications, such as preterm birth and ovarian hyperstimulation syndrome, are reduced (Evans *et al.*, 2014; Takeshima et al., 2016). Therefore, FET may be an efficient method of safely treating infertile couples at a relatively low cost.

However, the effects of the endometrium preparation method on the outcomes of pregnancies conceived with FET are relatively unclear (Wennerholm *et al.*, 2013; Ishihara *et al.*, 2014; Opdahl *et al.*, 2015). While several studies have investigated the rates of pregnancy, live birth, or miscarriage, the results remain controversial, and the best method of preparing the endometrium for embryo transfer remains unknown (Groenewoud *et al.*, 2013). Basically, embryos are transferred to the endometrium prepared by either normal ovulation or hormonal replacement with estradiol and progesterone. Because the preparation of the endometrium with hormonal replacement requires medication, this condition might be less 'physiological' than a natural ovulatory cycle (Groenewoud *et al.*, 2013). During the implantation period, progestin induces decidualization of estradiol-primed human endometrial stromal cells. It also assists with extravillous trophoblast (EVT) invasion and vascular remodeling through changes in the decidual cell-derived regulators of hemostasis, fibrinolysis, extracellular matrix turnover, and vascular tone (Schatz *et al.*, 2016). This tightly controlled EVT invasion is essential for pregnancy because aberrance or defects in EVT invasion can lead to obstetrical complications such as pre-eclampsia and placenta accreta (Esh-Broder et al., 2011; Chen et al., 2012; Schatz *et al.*, 2016). However, besides pregnancy and live birth rates, the associations between the method of endometrium preparation and other pregnancy outcomes, including obstetrical complications, have hardly been investigated.

We recently investigated the Japanese assisted reproductive technology (ART) registry database and reported the increased incidence of cesarean section and post-term delivery after FET during a hormonal replacement cycle (HRC-FET) compared with FET during a natural ovulatory cycle (NC-FET) (Saito *et al.*, 2017b). While the details of the pathophysiological conditions underlying these differences were not previously addressed, these groups of patients may harbor different risks of obstetrical outcomes. Because estrogen and progesterone are indispensable for normal placental development, altered levels of these hormones might lead to placenta-related complications such as hypertensive disorders of pregnancy (HDP), placenta accreta, placenta previa, and placenta abruption (Esh-Broder et al., 2011; Kaser et al., 2015; Schatz *et al.*, 2016). In this regard, gestational diabetes mellitus (GDM) might also be associated with the placenta because the placenta produces insulin-counteracting hormones and causes physiological insulin resistance during pregnancy (Brănişteanu and Mathieu, 2003).

To clarify the differences in the pregnancy outcomes between patients who undergo HRC-FET and NC-FET, we statistically analyzed the Japanese national ART registry database.

Materials and Methods

Data source

This study was approved by the institutional review board and the registration and research subcommittee of the Japan Society of Obstetrics and Gynecology (JSOG) Ethics Committee. The ART registry data set was provided by JSOG according to the guidelines determined. These data were collected on a mandatory basis from every ART clinic in Japan through an online system. Because the Japanese government only subsidizes patients who are treated at JSOG-accredited ART clinics, all the ART clinics in Japan participate in the registry, and the quality of the data are policed. Cycle-specific information of each treatment cycle, including patients' age, endometrium preparation method, and pregnancy outcomes were recorded separately by ART clinics. Treatment cycles from one patient were not linkable and data regarding previous treatments were not available. The data included in this study were obtained from ART cycles at 574 ART clinics in 2014. Since the use of donated gametes and embryos for ART is forbidden in Japan, autologous embryos were transferred in all cases (Saito *et al.*, 2018).

A total of 393 745 ART cycles were recorded in the ART registry database (Fig. 1). We excluded cycles where fresh embryos (n = 236 516) and frozen-thawed oocytes (n = 187) were used for the treatment. Among the FET cases, cycles with ovarian stimulation (n = 8419) and missing or incomplete data for protocols on the preparation of the endometrium (n = 43 389) were excluded. The remaining cases were categorized into an NC-FET group (NC group,

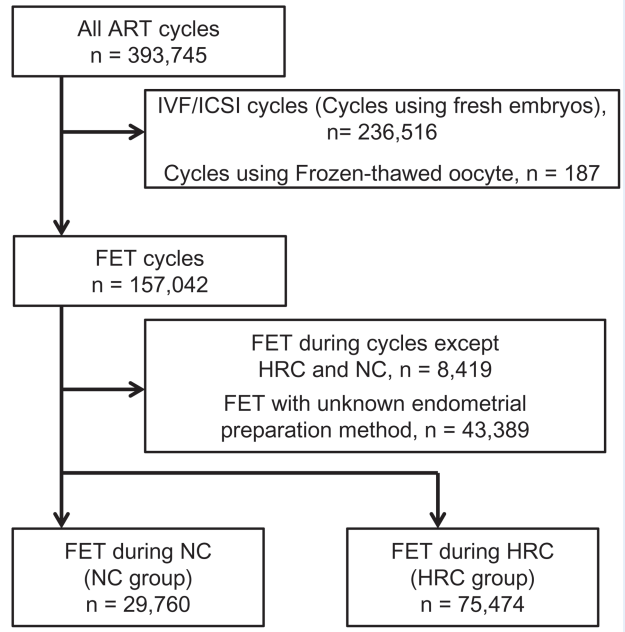

Figure 1 A flow diagram showing the distribution of the study populations. IVF, in-vitro fertilization; ICSI, intracytoplasmic sperm injection; HRC, hormone replacement cycle; NC, natural ovulatory.

n = 29 760) and an HRC-FET group (HRC group, n = 75 474) and subjected to the analyses below. The NC-FET group included so-called modified natural FET cycles where hCG trigger or luteal support was provided (Casper and Yanushpolsky, 2016).

In Japan, the choice of endometrial preparation method is made based on the patients' condition and the treatment policy of respective ART centers or physicians. For instance, patients with ovulation disorders or impaired endometrium development often undergo HRC-FET, because these patients have trouble preparing the endometrium with natural ovulation. On the other hand, HRC-FET is also chosen due to the convenience of scheduling the date of FET. As no consensus has been reached on the optimal protocol to manage HRC, the management totally depends on each clinic or physician. Regarding the route of administration, oral and transdermal estrogen preparations and oral, injectable and transvaginal progesterone preparations are used. The route, dosage, and duration of the administration are usually determined by reference to the physiological hormonal status, while the management protocol differs among clinics. The hormone levels are measured at physicians' discretion, but such data were not available because of the inherent nature of the database. The Japan Society for Reproductive Medicine recommends FET of four-cell embryos, eight-cell embryos, and blastocysts for the second, third, and fifth days after ovulation, respectively.

Definition of pregnancy outcomes

The primary outcome was the rate of obstetrical complications. Obstetrical complications included placenta previa, placenta abruption, placenta accreta, HDP, preterm premature rupture of the membrane, GDM, and uterine inversion. HDP in this study includes preeclampsia and gestational hypertension and excludes chronic hypertension. GDM is diagnosed based on recommendations by the international association of diabetes and pregnancy study groups (International Association of Diabetes and Pregnancy Study Groups Consensus Panel, 2010). As secondary outcomes, we analyzed the rate of pregnancy, live birth, cesarean section, and pre- and post-term delivery and outcomes of the offspring, such as sex and weight at birth. In this study, pregnancy is diagnosed by detecting the gestational sac with ultrasonography. Gestational age was divided into delivery before 37 gestational weeks (preterm delivery), from 37 to 41 weeks (term delivery) and after 41 weeks (post-term delivery). Neonatal birth weight was divided into <2500 g, between 2500 and 3999 g, and ≥4000 g. In addition, we investigated the neonatal birth weight regarding small- and large-for-gestational age neonates according to the new standard of average size and weight of newborns set by the Japan Pediatric Society (Itabashi *et al.*, 2010). Cases with anomalous data for gestational age and weight at birth were excluded from the analysis using the criteria in the report of the United States national reference for fetal growth (Alexander *et al.*, 1996).

Statistical analyses

For all characteristics, we calculated the mean and standard deviation values for continuous variables and the number of the cases for discrete variables for both the HRC and NC groups. Differences between the groups were evaluated by the chi-squared test for dichotomous variables and the Mann–Whitney *U*-test for continuous variables. The pregnancy rate was calculated using the number of FET cycles as the denominator. Live birth, miscarriage, and stillbirth rates were calculated using the number of successful pregnancies as the denominator. The rates of pregnancy outcomes, including complications and cesarean delivery, were calculated as the proportion among the total number of births (live birth and stillbirth) after 22 weeks of gestation for both groups, and the differences between the two groups were evaluated by the chi-squared test.

The crude and adjusted odds ratios (cOR and aOR, respectively) of HRC-FET compared with NC-FET for pregnancy outcomes and obstetrical complications were evaluated by multiple logistic regression analysis. Considering that many comparisons were made, we corrected the confidence intervals (CIs) using the Bonferroni method according to the number of the outcomes. Covariates included in all models were method of endometrium preparation (HRC vs NC), maternal age, the number of the embryos transferred, embryo stage at transfer (cleaved embryo versus blastocyst), use of assisted hatching, and indications for ART. We did not include the method of luteal-phase support for the models because the hormonal status was totally different in the HRC and NC groups, even when they were supported by the same method. In addition, the number of the offspring was omitted because of its multicollinearity with the number of the embryos transferred. All covariates were considered independent variables in the model and were included as either a continuous or a dichotomous variable. For the sensitivity analysis, we repeated our analysis restricted to singleton cases and singleton live birth cases. For this analysis, we also evaluated the differences in the offspring's outcomes between pregnancies induced by HRC-FET and those by NC-FET. However, we did not evaluate the differences in the offspring's outcomes in the main analysis, which included multiple births, as outcomes of births from the same mother would not be independent from each other in multiple birth cases.

All statistical analyses were performed using the SPSS software program, version 22.0 (SPSS, Chicago, IL, USA). The significant difference was defined as $P < 0.05$.

Results

Table I shows the characteristics and pregnancy outcomes of the study populations. In the HRC group, the average maternal age was lower, number of transferred embryos was higher, cleavage stage embryos were more often transferred, and assisted hatching was more often performed than in the NC group. Regarding the indications for ART, the proportions of endometriosis and male factor were higher in the HRC group than in the NC group, while the proportions of tubal factor and unexplained infertility were lower. In the category 'others', the frequently recorded ART indications were advanced maternal age and ovulation disorder. The pregnancy rate was significantly lower in the HRC group than in the NC group (32.1% vs 36.1%).

The obstetrical and offspring's outcomes among all deliveries (stillbirths and live births) at ≥22 weeks of gestation are shown in Table II.

Table I The characteristics and pregnancy outcomes of the NC and HRC groups.

	NC group (n = 29 760)	HRC group (n = 75 474)	*P*-value
Maternal age, years	38.0 ± 4.1	37.2 ± 4.4	<0.001
Number of embryos transferred	1.09 ± 0.35	1.21 ± 0.47	<0.001
Stage at embryo transfer			
Blastocyst	22 503 (75.6%)	48 326 (64.0%)	<0.001
Cleavage	6446 (21.7%)	23 602 (31.3%)	<0.001
Cycles where ET was canceled	413 (1.4%)	1152 (1.5%)	0.095
Others	256 (0.9%)	2047 (2.7%)	<0.001
Unknown	142 (0.5%)	347 (0.5%)	
Assisted hatching			<0.001
Performed	17 838 (59.9%)	52 030 (68.9%)	
Not performed	11 780 (39.6%)	23 097 (30.6%)	
Unknown	142 (0.5%)	347 (0.5%)	
Luteal-phase support			
None	4868 (16.4%)	1530 (2.0%)	<0.001
Progesterone alone	13 906 (46.7%)	7402 (9.8%)	<0.001
hCG	2421 (8.1%)	77 (0.1%)	<0.001
Progesterone + hCG	3893 (13.1%)	1081 (1.4%)	<0.001
Estrogen + progesterone	3272 (11.0%)	61 561 (81.6%)	<0.001
Estrogen + progesterone + hCG	667 (2.2%)	2294 (3.0%)	<0.001
Others	590 (2.0%)	1185 (1.6%)	<0.001
Unknown	143 (0.5%)	344 (0.5%)	0.614
Indication for ART			
Tubal factor	5054 (17.0%)	12 191 (16.2%)	0.001
Endometriosis	1710 (5.7%)	5730 (7.6%)	<0.001
Antisperm antibody-positive	137 (0.5%)	395 (0.5%)	0.210
Male factor	7142 (24.0%)	22 551 (29.9%)	<0.001
Unexplained infertility	15 978 (53.7%)	29 815 (39.5%)	<0.001
Others	3368 (11.3%)	20 850 (27.6%)	<0.001
Pregnancy			<0.001
Yes	10 755 (36.1%)	24 225 (32.1%)	
No	18 854 (63.4%)	50 904 (67.4%)	
Unknown	151 (0.5%)	345 (0.5%)	
Number of the offspring	(n = 10 755)	(n = 24 225)	<0.001
Singleton	10 507 (97.7%)	23 337 (96.3%)	
Twin	242 (2.3%)	872 (3.6%)	
Triplet	6 (0.1%)	16 (0.1%)	

Data are expressed as the mean ± standard deviation or number (%).
Differences between the groups were evaluated by the Mann–Whitney *U*-test or chi-squared test.

Among pregnancies, live birth rates were lower (67.1% vs 71.9%), and the miscarriage rate was higher (28.7% vs 25.0%) for pregnancies conceived with HRC-FET than for those conceived with NC-FET. The rates of stillbirth, induced abortion and ectopic pregnancy were comparable between the two groups. Both pre- and post-term births as well as cesarean section were observed more frequently among pregnancies due to HRC-FET than among those due to NC-FET. HDP, placenta accreta, preterm premature rupture of the membrane

Table II The pregnancy outcomes of the cases in the NC and HRC groups.

	NC group	HRC group	*P*-value
Outcome of pregnancy	(n = 10 755)	(n = 24 225)	
Live birth[a]	7737 (71.9%)	16 248 (67.1%)	<0.001
Stillbirth	26 (0.2%)	74 (0.3%)	0.330
Miscarriage	2686 (25.0%)	6952 (28.7%)	<0.001
Induced abortion	48 (0.4%)	103 (0.4%)	0.791
Ectopic pregnancy	48 (0.4%)	154 (0.6%)	0.032
Unknown	210 (2.0%)	694 (2.9%)	<0.001
Obstetrical outcomes of live birth and stillbirth cases	(n = 7763)	(n = 16 322)	
Gestational age at birth, weeks	38.5 ± 1.9	38.6 ± 2.2	<0.001
Gestational age category			<0.001
≤36 weeks	579 (7.4%)	1441 (8.8%)	
37–41 weeks	6931 (89.4%)	14 126 (86.5%)	
≥42 weeks	24 (0.3%)	151 (0.9%)	
Unknown	229 (2.9%)	604 (3.7%)	
Mode of delivery			<0.001
Vaginal delivery	4917 (63.4%)	8533 (52.3%)	
Cesarean section	2617 (33.7%)	7271 (44.5%)	
Unknown	229 (2.9%)	518 (3.2%)	
Obstetrical complications			
Placenta previa	66 (0.9%)	119 (0.7%)	0.343
Placenta abruption	6 (0.1%)	26 (0.2%)	0.129
HDPs	237 (3.0%)	656 (4.0%)	<0.001
Placenta accreta	11 (0.1%)	142 (0.9%)	<0.001
GDM	256 (3.3%)	249 (1.5%)	<0.001
pPROM	15 (0.2%)	70 (0.4%)	0.005
Uterine inversion	0 (0.0%)	16 (0.1%)	0.005
Outcomes of the offspring in live birth and stillbirth cases	(n = 7974)	(n = 16 947)	
Sex of offspring			0.001
Male	4014 (50.3%)	8538 (50.4%)	
Female	3792 (47.6%)	7917 (46.7%)	
Unknown	168 (2.1%)	492 (2.9%)	
Birth weight, g	2991.7 ± 476.5	2996.4 ± 535.0	0.493
Birth weight category			<0.001
<2500g	860 (10.8%)	2209 (13.0%)	
2500–3999g	6859 (86.0%)	13 974 (82.5%)	
≥4000g	77 (1.0%)	271 (1.6%)	
Unknown	178 (2.2%)	493 (2.9%)	
	(n = 7681)	(n = 15 967)	
Small for gestational age	525 (6.8%)	1176 (7.4%)	0.147
Large for gestational age	944 (12.3%)	2163 (13.5%)	0.008

pPROM, preterm premature rupture of the membrane.
Data are expressed as the mean ± standard deviation or number (%).
Differences between the groups were evaluated by the Mann–Whitney *U*-test or chi-squared test.
[a]Live birth cases include three selective reduction cases in NC group, eight selective reduction cases, and one heterotopic pregnancy case in the HRC group.

(pPROM) and uterine inversion were also more often reported in the HRC group. In contrast, the incidence of GDM was significantly lower in the HRC group than in the NC group. Regarding the offspring's outcome, the average birth weight was comparable between the two groups. However, the proportions of infants with birth weight <2500 g and ≥4000 g, as well as those large-for-gestational age, were higher in the HRC group than in the NC group.

Through the multiple logistic regression model, we found that HRC was an independent risk factor for cesarean section (aOR, 1.69; 95% CI, 1.55–1.84), post-term delivery (aOR, 3.28; 95% CI, 1.73–6.19), HDP (aOR, 1.43; 95% CI, 1.14–1.80), and placenta accreta (aOR, 6.91; 95% CI, 2.87–16.66) (Table III). In contrast, the risk of GDM was significantly reduced in the HRC group (aOR, 0.52; 95% CI, 0.40–0.68).

For the sensitive analysis, we restricted the analysis to singleton cases (Supplementary Table SI). In this analysis, HRC remained significantly associated with increased risks of cesarean section (aOR, 1.75; 95% CI, 1.60–1.92), post-term delivery (aOR, 3.25; 95% CI, 1.67–6.32), HDP (aOR, 1.45; 95% CI, 1.14–1.85), and placenta accreta (aOR, 6.53; 95% CI, 2.60–16.42) and a decreased risk of GDM (aOR, 0.52; 95% CI, 0.39–0.69). We also found that among singleton cases, the risks of birth weight ≥4000 g (aOR, 1.76; 95% CI, 1.18–2.62) and being large for gestational age (aOR, 1.18; 95% CI, 1.03–1.34) were higher among pregnancies from HRC-FET than among those from NC-FET. These findings were reproducible in the analyses of singleton live birth cases (Supplementary Table SII).

Discussion

In our large retrospective cohort study of over 100 000 FET cycles, we demonstrated for the first time that HRC-FET is significantly associated with increased risks of HDP and placenta accreta and a decreased risk of GDM compared with NC-FET. We also confirmed the previous finding that pregnancies conceived by HRC-FET had an increased incidence of cesarean and post-term delivery (Saito et al., 2017).

While previous studies have reported an increased incidence of HDP and placenta accreta among pregnancies after FET (Ishihara et al., 2014; Kaser et al., 2015), the detailed mechanisms underlying this difference remains unknown. Regarding this point, we demonstrated an association between endometrium preparation by hormonal replacement and HDP and placenta accreta. This result suggests that endometrial preparation methods are associated with the later development of obstetrical complications. Known risk factors of HDP are advanced maternal age and multiple pregnancies (Mol et al., 2016). However, in the present study, the average maternal age in the HRC group was significantly lower than that in the NC group. In addition, the odds of HRC against NC for HDP remained increased in the sensitivity analysis restricted to cases of singleton births (Supplementary Table S1). In line with the increased odds of HDP and placenta accreta after HRC-FET demonstrated by a multiple logistic regression analysis (Table III), these results also support the association between endometrium preparation with hormonal replacement and HDP. A history of uterine surgery and placenta previa are significant risk factors for placenta accreta. Although the history of uterine surgery was not included in the analysis, we found that the frequency of placenta previa was comparable between the two groups. Therefore, the increased incidence of placenta accreta in this study does not appear to have been caused by an increased incidence of placenta previa.

Given that HRC requires medication and is a less 'physiological' condition (Groenewoud et al., 2013), it may modulate the risk of obstetrical complications through changes in the endometrial condition and subsequent placental development. Regarding the hormonal status, there is no consensus on the optimum hormonal levels during HRC and the duration of hormonal replacement after implantation (Groenewoud et al., 2013). Because progesterone induces decidualization of the endometrial stromal cells and regulates EVT invasion, aberrant progesterone levels in early pregnancy can lead to

Table III Crude and adjusted ORs of a hormone replacement cycle against a natural ovulatory cycle for obstetrical outcomes.

Outcome	Crude OR (95% CI)[b]	Adjusted OR[a] (95% CI)[b]
Cesarean section	**1.60 (1.48–1.74)**	**1.69 (1.55–1.84)**
Preterm delivery	**1.21 (0.96–1.30)**	**1.12 (1.05–1.40)**
Post-term delivery	**3.04 (1.65–5.59)**	**3.28 (1.73–6.19)**
Obstetrical complications		
Placenta previa	0.86 (0.56–1.31)	1.00 (0.64–1.56)
Placenta abruption	2.06 (0.59–7.24)	1.58 (0.43–5.88)
HDPs	**1.33 (1.07–1.65)**	**1.43 (1.14–1.80)**
Placenta accreta	**6.19 (2.60–14.73)**	**6.91 (2.87–16.66)**
GDM	**0.45 (0.35–0.58)**	**0.52 (0.40–0.68)**
Preterm premature rupture of the membrane	1.94 (0.87–4.31)	1.87 (0.82–4.28)

OR, odds ratio; CI, confidence interval.
Odds ratios were obtained via a multiple logistic regression analysis.
Significantly increased or reduced odds are indicated by boldface.
[a]Adjusted for maternal age, embryo stage at transfer, number of the embryos transferred, use of assisted hatching, and indications for assisted reproductive technology.
[b]95% CIs are corrected using the Bonferroni correction according to the number of the outcomes examined (9 outcomes; alpha value, 0.0056).

over-invasion or an invasion defect of the EVT (Chen *et al.*, 2012). Indeed, Tamimi *et al.* (2003) reported an association between an increased serum progesterone level in the early third trimester with the later development of pre-eclampsia, in which the placenta typically shows features of superficial placentation. In contrast, a decreased progesterone level in the early pregnancy can lead to placenta accreta through failure of normal decidualization and over-invasion of EVT (Jauniaux and Jurkovic, 2012; Saito *et al.*, 2018). Regarding this point, uterine inversion, which was exclusively reported in the HRC group (Table II), also deserves attention because placental attachment is a significant risk factor for this complication (Mirza and Gaddipati, 2009). The increased incidence of uterine inversion after HRC-FET in this study may support an association between HRC and the abnormal adherence of the placenta to the uterine wall. Less-frequent complications such as placenta accreta and uterine inversion are catastrophic events and often require intensive care, including hysterectomy (Mirza and Gaddipati, 2009; Jauniaux and Jurkovic, 2012). Furthermore, these complications are sometimes difficult to predict and are often diagnosed at the time of delivery. Therefore, our findings may help improve the obstetrical management by indicating patients who may need additional care for placenta accreta and uterine inversion, as well as HDP.

Notably, we found that the risk of GDM after HRC-FET was almost half of that after NC-FET. While GDM is associated with maternal characteristics, such as age, obesity, diabetes in the family, and ethnicity (Reece *et al.*, 2009), our results showed that ART treatment also modulated the risk of GDM. Ashrafi *et al.* (2014) reported that the risk of GDM was two-fold higher in women with pregnancies conceived after ART than in those who conceived spontaneously. They also reported progesterone use during pregnancy as an important risk factor for GDM (Ashrafi *et al.*, 2014). During pregnancy, physiological insulin resistance is induced by insulin-counteracting hormones produced by the placenta, such as progesterone and human placental lactogen (Brănişteanu and Mathieu, 2003). Therefore, the robust development of the placenta is essential for this physiological insulin resistance. Indeed, Gauster *et al.* (2012) reported that placentas from mothers with GDM showed anatomical and physiological alterations compared with those from mothers without GDM. Because the rate of placenta-related diseases increased in patients after HRC-FET in this study, the risk of GDM might be also modulated through anatomical or functional changes in the placenta. Specifically, the decreased secretion of insulin-counteracting hormones from the placenta might suppress the pathogenesis of GDM in some HRC-FET-derived pregnancies. Because the hormonal status and placenta were not investigated in this study, this notion warrants further study to clarify a possible association between the hormonal status during the peri-implantation period and the later development of GDM.

In this study, we found a slightly lower rate of pregnancy as well as a lower live birth rate among successful pregnancies after HRC-FET than after NC-FET. While previous studies exploring the optimum endometrium preparation for FET obtained controversial results (Groenewoud *et al.*, 2013), our study adds some evidence supporting a better pregnancy and live birth rate after NC-FET than after HRC-FET. However, considering that both NC-FET and HRC-FET achieved a pregnancy rate of more than 30% in this study, both methods seem to be reasonable. An ovulation disorder is a good indication for HRC-FET, as such patients will experience difficulty preparing the endometrium naturally. In addition, HRC-FET reduces the need for repeated hospital visits and enables patients with or without ovulation disorders to schedule FET at their leisure (Groenewoud *et al.*, 2013). In fact, the large numbers of HRC-FET cycles reporting tubal factor, male factor and unexplained infertility in Table I imply that many patients who ovulate naturally undergo HRC-FET. The above merits of HRC-FET might compensate for the slight decreased rates of pregnancy and live birth observed in this study. Nevertheless, we should also consider obstetrical risks when we decide on the endometrium preparation method, since HRC-FET and NC-FET patients harbor different risks for obstetrical complications and cesarean delivery, as we found in this study.

The strength of this study is its large sample size. The Japanese ART registry, one of the largest ART registries in the world, covers virtually all ART cycles performed in Japan. In order to investigate relatively infrequent events, like minor obstetrical complications, a large sample size is essential. In this sense, our sample size was large enough to assess the risk of various obstetrical complications. However, several limitations associated with this study warrant mention. First, our data included all ART cycles in Japan, and the HRC and NC groups differed considerably in many parameters, including size. On the other hand, selection bias had very little effect on the results of this study as there was no process to select samples. Therefore, the lack of selection bias compensates for the shortcomings caused by the differences in the two groups. Second, while patient characteristics, such as socioeconomic status, smoking habit, alcohol consumption, duration of infertility, parity, body mass index, and embryo quality, likely influence pregnancy outcomes, we did not obtain any data on these parameters because of the inherent limitations in the ART registry. These unmeasured characteristics possibly affect the decision to choose the method of endometrial preparation, and our results may therefore be confounded by this susceptibility bias. For the same reason, we lacked data on previous ART attempts or pregnancies, facilities for treatment, and reason why HRC-FET was chosen over NC-FET, so we could not analyze what kinds of women were prone to receive HRC-FET either. Since endometrial thickness in the fresh cycle is associated with that in the FET cycle (Jimenez *et al.*, 2013), data regarding previous treatment could potentially influence the decision made regarding endometrial preparation in the FET. In addition, factors driving the decision to choose HRC, as well as HRC itself, can be associated with obstetrical complications. For example, polycystic ovarian syndrome, a common ovulation disorder that would give good reason to use HRC, is associated with several obstetrical complications (Palomba *et al.*, 2015). Therefore, the reason for choosing HRC deserves attention because it is also associated with the risk of obstetrical complications and can possibly distort the results of the analyses. In this regard, lower body mass index associates with both impaired endometrial development and decreased risk of GDM. If there were more patients with lower body mass index in the HRC group, it would also explain the reduced risk of GDM in this group. However, increased risk of large-for-gestational age offspring in the HRC group argues against this presumption, because large-for-gestational age offspring is normally associated with maternal obesity (Sridhar *et al.*, 2013). This inconsistency regarding body mass index and pregnancy outcomes warrants further attention. Ideally, future prospective studies that include these factors should be conducted. Nevertheless, our results are still worth noting because we could demonstrate that patients conceiving with

HRC-FET had a higher risk of obstetrical complications, suggesting that such women may need closer monitoring than those conceiving with NC-FET.

In conclusion, this is the first study to demonstrate an association between the endometrium preparation method and obstetrical complications. We analyzed Japanese ART registry data and found that the risks of HDP and placenta accreta were higher and the risk of GDM was lower in patients conceiving after HRC-FET than in those conceiving after NC-FET. Further studies that include other possible confounders are required to clarify the mechanism underlying the association between endometrium preparation and obstetrical complications.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Acknowledgements

The authors express their gratitude to JSOG for kindly providing the data and also thank all of the clinics that participated in the Japanese ART registry for their continuous support in the data collection.

Authors' roles

K.S., A.K., and H.S. contributed to the study conception and design. K.S., A.K., T.I., N. Morisaki, M.M., and K.M. participated in the analysis of the data. K.S., M.F., N. Miyasaka, O.I., M.I., and H.S. participated in the interpretation of the results. K.S. and H.S. drafted the manuscript. All of the authors have critically reviewed the manuscript and approved the final version.

Funding

No funding was obtained for this work.

Conflict of interest

The authors declare no conflicts of interest in association with the present study.

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