Endometrial preparation and maternal and obstetrical outcomes after frozen blastocyst transfer

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BACKGROUND: Two types of endometrial preparation protocols are used for frozen embryo transfers in current practice: hormone replacement and the natural cycle. Endometrial preparation in the natural cycle reportedly increases the chances of live birth and decreases early pregnancy loss compared with that in the hormone replacement cycle. However, the influence of endometrial preparation on maternal and neonatal health remains unclear.

OBJECTIVE: This study aimed to investigate whether the differences between hormone replacement cycle and natural cycle influence perinatal outcomes and risk of congenital anomalies in frozen-thawed blastocyst transfer fetuses or births.

STUDY DESIGN: Perinatal outcomes and congenital abnormalities were compared between the natural and hormone replacement cycles. According to the timing of ovulation, frozen-thawed blastocyst transfers in the natural cycle were classified into 2 patterns: on day 4.5 (ovulation 4.5) or day 5 (ovulation 5.0) after ovulation. When the serum luteinizing hormone level was not increased on the day of the trigger, a single vitrified-warmed blastocyst transfer was performed on day 7 after the trigger (ovulation 5.0). When the luteinizing hormone level was slightly increased on the day of trigger, single vitrified-warmed blastocyst transfer was performed on day 6 after the trigger (ovulation 5.0). In total, 67,018 cycles (ovulation 4.5, 29,705 cycles; ovulation 5.0, 31,995 cycles; hormone replacement, 5318 cycles) of frozen-thawed blastocyst transfer between January 2008 and December 2017 at Kato Ladies Clinic were retrospectively analyzed. During the study period, embryo cryopreservation was performed using a vitrification method in all cycles.

RESULTS: Hormone replacement cycles were associated with a higher occurrence of hypertensive disorders of pregnancy (adjusted odds ratio, 2.16; 95% confidence interval, 1.66−2.81) and placenta accreta (adjusted odds ratio, 4.14; 95% confidence interval, 1.64−10.44) compared with the natural cycle. The risks of cesarean delivery (adjusted odds ratio, 1.93; 95% confidence interval, 1.78−2.18), preterm birth (adjusted odds ratio, 1.55; 95% confidence interval, 1.25−1.93), and low birthweight (adjusted odds ratio, 1.42; 95% confidence interval, 1.18−1.73) were also higher for hormone replacement cycles. No significant difference in the risk of congenital anomalies was observed between the 2 cycles.

CONCLUSION: The risk of hypertensive disorders of pregnancy, placenta accreta, cesarean delivery, preterm delivery, and low birthweight was higher in hormone replacement cycles than in natural cycles, whereas the risk of congenital anomalies was similar between both cycles. Further follow-up is needed to investigate these risks and to explore alternative endometrial preparation methods.

Key words: clomiphene citrate, congenital anomalies, frozen blastocyst transfer, hormone replacement cycle, natural cycle, pregnancy complications

Introduction
The number of frozen embryo transfer (FET) cycles has progressively increased since vitrification techniques were established in 1999 and 2000.1,2 In Japan, 89.4% of births derived from assisted reproductive technologies (ARTs) are obtained from FET cycles.3 FET has been enhanced by improvements in the field of cryopreservation and the treatment strategy of elective single-embryo transfers to limit multiple pregnancies.4 Using the cryopreservation technique, embryos can be transferred at the optimal time without the detrimental effects that ovarian stimulation exerts on endometrial function; therefore, improved pregnancy
outcomes are expected after FETs compared with those after fresh embryo transfers.5–7

Uterine receptivity and synchronization between the endometrium and embryo are essential for successful embryo implantation8; thus, FET should be performed when both the embryo and endometrium are well-prepared. Two types of endometrial preparation protocols are currently used for FETs: hormone replacement (HR) cycle (an artificial cycle) and the natural cycle. The HR cycle is the most commonly used protocol worldwide because it enables flexibility in scheduling the embryo transfers for in vitro fertilization (IVF) centers. However, because endometrial preparation in the HR cycle requires long-term hormonal administration, a greater physical and financial burden is imposed on patients. By contrast, endometrial preparation in the natural cycle needs the least medication; therefore, the physical and financial demand can be reduced despite the inflexibility in scheduling. Recent studies have suggested that endometrial preparation in the natural cycle may improve live birth and decreases early pregnancy loss compared with the HR cycle9–11; therefore, natural-cycle FETs could be considered an effective strategy to improve live-birth rates. Furthermore, in terms of maternal outcomes, FETs in the HR cycle are reportedly associated with an increased occurrence of hypertensive disorders of pregnancy (HDP), postpartum hemorrhage, placenta accreta, and gestational diabetes mellitus compared with natural cycles.12–16 However, these reports lack key information such as the protocol for endometrial preparation in both natural and HR cycles and grade of transferred embryos. Furthermore, the number and stage of transferred embryos were significantly different between the protocols. These factors may result in critical bias when investigating maternal and infant health.

Thus, this study aimed to compare maternal and obstetrical outcomes between the natural and HR cycles under uniform protocols. Furthermore, the outcomes in the natural cycle were stratified by the timing of the transfers and compared to reveal whether the transfer timing affected maternal and obstetrical outcomes. In this study, we retrospectively analyzed maternal and obstetrical outcomes in a single-center 10-year cohort after single vitrified-warmed blastocyst transfers (SVBTs) in natural and HR cycles.

Materials and Methods

Study patients

We retrospectively analyzed all clinical records of women who underwent oocyte retrieval during a clomiphene citrate (CC)-based minimal stimulation cycle followed by SVBT in the natural and HR cycles at the Kato Ladies Clinic between January 2008 and December 2017. Only data from patients with singleton pregnancies were included. During the study period, embryo cryopreservation was performed using a vitrification method in all cycles. Complete follow-up data from patients with delivery were used for the analysis of pregnancy complications, and patients with incomplete data were excluded. Follow-up data on obstetrical outcomes were compared among the groups. Data of patients who had cervical incompetence were excluded from the analysis of neonatal outcomes. We classified infertility according to its cause as ovulation (irregular menstruation caused by polycystic ovarian syndrome or diminished ovarian reserve [DOR]), tubal factor (diagnosed by hysterosalpingography), endometriosis (diagnosed by ultrasound), endometrial factor (diagnosed by hysteroscopy), male factor (diagnosed by semen analysis), combined, or unexplained (patients not diagnosed with any cause).

Ethical approval

This retrospective cohort study was approved by the Institutional Review Board of Kato Ladies Clinic (approval number: 21-14). Written informed consent for the analysis was obtained from all patients.

Minimal ovarian stimulation cycle in vitro fertilization

A detailed protocol for minimal stimulation with CC has been previously reported.17–19 Briefly, CC (50–100 mg/d; Fuji Pharma Co, Ltd, Tokyo, Japan) was orally administered from day 3 of the retrieval cycle to the day before induction of final oocyte maturation (days 9–21). Ovulation triggering was performed using a nasal spray containing the gonadotropin-releasing hormone agonist buserelin (Suprecur; Mochida Pharmaceutical Co, Ltd, Tokyo, Japan; or Buserelin; Fuji Pharma Co, Ltd, Tokyo, Japan).

Oocyte retrieval is generally performed 34 to 36 hours after triggering using a 21–22G needle (Kitazato Corporation, Shizuoka, Japan) without anesthesia or follicular flushing. Cumulus–oocyte complexes were collected, washed, and then transferred to a
human tubal fluid medium (Kitazato Corporation) with paraffin oil at 5% CO₂ in air at 37°C for culturing until either conventional IVF was performed 3 hours later,20 or in cases of intracytoplasmic sperm injection, denudation was performed 4 hours after oocyte retrieval.21 All embryos were cultured at 37°C (gas phase: 5% O₂, 5% CO₂, and 90% N₂), with 100% humidity in a water jacket or with nonhumidified incubators (Astec Co, Ltd, Fukuoka, Japan). Embryo vitrification and warming were performed using Cryotop (Kitazato Corporation), as described previously.22 Briefly, blastocysts were equilibrated in equilibrium solution consisting of 7.5% (v/v) ethylene glycol and 7.5% (v/v) dimethylsulphoxide for 15 minutes. Blastocysts were then transferred to a vitrification solution consisting of 15% (v/v) ethylene glycol, 15% (v/v) dimethylsulphoxide, and 0.5 M sucrose for 1.5 minutes. Next, they were placed on the Cryotop and immediately plunged into liquid nitrogen. For warming, the Cryotop was placed into a warming solution of 1.0 M sucrose at 37°C for 1 minute. The blastocysts were then removed from the warming solution and transferred to a diluted solution of 0.5 M sucrose at room temperature. After 3 minutes, they were transferred to the washing solution without sucrose. For final dilution, the blastocysts were transferred to the washing solution for 1 minute.

**Embryo transfer**

The endometrial preparation method was determined after consultation with patients at the initiation of SVBT cycles. In principle, SVBT was performed after confirmation of spontaneous ovulation in the natural cycle. Alternatively, HR cycles were selected in cases of severe ovulatory failure, ovarian insufficiency, luteal insufficiency, or other cases in which SVBT in the ovulatory cycle was deemed inappropriate.

In the natural cycle, ovulation date was estimated from the endometrial thickness, follicle size, and hormonal patterns, or determined by performing human chorionic gonadotropin (hCG) triggering. The timing of SVBT in the natural cycle was classified into 2 patterns according to the timing of ovulation: on day 4.5 (OV 4.5) or day 5 (OV 5.0) after ovulation. When the serum luteinizing hormone (LH) level had not increased on the day of the trigger, SVBT was scheduled on day 7 after the trigger (OV 5.0). When the LH level was slightly increased on the day of trigger, SVBT was scheduled on day 6 after the trigger (OV 5.0). SVBTs were performed as previously described.24 Embryo transfer was performed under vaginal ultrasound guidance using a specially designed soft silicone inner catheter (Kitazato ET catheter; Kitazato Corporation) by placing a single embryo in a minimal volume in the upper part of the uterine cavity. Dydrogesterone (Duphaston, 30 mg/d; Mylan EPD G.K., Tokyo, Japan) was administered orally during the early luteal phase after SVBT.

The HR cycle consists of administration of exogenous estradiol and progesterone. Estradiol administration (Estrana, Hisamitsu Pharmaceutical, Co, Inc, Saga, Japan; Julina, Bayer Yakuhin, Ltd, Tokyo, Japan) was initiated on the second day of menstruation. Dydrogesterone administration was initiated after confirmation that the endometrial thickness was >7 mm and serum estradiol level had reached 200 pg/mL. Progesterone was intravaginally administered from 3 days after the initiation of dydrogesterone administration. SVBTs were performed 4 days after the initiation of progesterone administration.

Maternal and neonatal outcomes were obtained from a questionnaire filled out by patients after the infant’s 1-month examination. Pregnancy complications were diagnosed in maternity hospitals. All pregnant women were invited to respond to the questionnaire at 9 weeks of gestation, at the second trimester, and after delivery. If they did not respond, we conducted a follow-up regarding their outcomes.

**Study outcomes**

The primary outcomes were pregnancy complications and major anomalies. Pregnancy complications include HDP; gestational diabetes mellitus; hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome; preterm premature rupture of membranes; low-lying placenta; placenta previa; placenta accreta; and placental abruption. Neonatal outcomes included gestational age (≤27, 28–36, and ≥37 weeks), birthlength, birthweight, small for gestational age (SGA), and large for gestational age (LGA).

The questionnaire requested information on the following: date and mode of delivery, sex, birthweight, birthlength, presence of any birth defect or other anomaly, and pregnancy complications. Live birth was defined as delivery at ≥22 weeks’ gestation. Preterm delivery was defined as delivery occurring at <37 weeks’ gestation. Low birthweight was defined as <2500 g. Perinatal mortality was defined as the sum of stillbirths (≥22 pregnancy weeks) and early (within 7 days) infant deaths. SGA and LGA were defined as birthweights <10th and >90th percentile, respectively, according to the Japanese national reference for neonates.25 Congenital anomalies were classified using the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) codes by reformattting the answers of the parent questionnaires.26 Only major congenital anomalies were classified according to the European Surveillance of Congenital Anomalies (EUROCAT) guidelines. Congenital anomalies were classified in the following 13 classes:27 nervous system, eyes, ears, face, neck, congenital heart defects, respiratory, oro-facial clefts, digestive system, abdominal wall defects, urinary, genital, limb, other anomalies or syndromes, and chromosomal. According to the EUROCAT guidelines revised in November 2021, the following cases were not registered: cases of cerebral palsy and cases with only minor defects, excluding cases that were associated with major anomalies.

**Statistical analyses**

All statistical analyses were performed using the JMP software (SAS Institute, Cary, NC). Proportion data were analyzed using the chi-square test. Normally distributed continuous parameters were compared using analysis of variance, and statistical significance was determined using Tukey’s test for post
hoch analysis. When the data were not distributed normally, the Kruskal–Wallis and the Steel–Dwass multiple-comparison test were used. A univariate logistic regression analysis was performed to identify confounders that were potentially associated with maternal and perinatal outcomes (Supplemental Table 1). The likelihood ratio test for the significance of the coefficient was performed, and variables with \( P < .10 \) were used as confounders. Similarly, a multivariate logistic regression analysis for maternal and perinatal outcomes was used to adjust bias (using covariates and confounders) and verify statistical significance using the Wald chi-square test.\(^2\) Odds ratios (ORs) and adjusted ORs (AORs) are reported with 95% confidence intervals for each group. The OV 4.5 group was used as the reference for the logistic regression analysis. A \( P \) value of \(< .05 \) was considered significant.

Results

Characteristics of the study cohort

In total, 67,018 SVBTs (OV 4.5 group, 29,705 cycles; OV 5.0 group, 31,995 cycles; HR group, 5318 cycles) were performed during the study (Table 1). Maternal age, body mass index, smoking history, number of previous deliveries, and causes of infertility were significantly different among the groups. Culture time and morphology of transferred blastocysts varied among the groups. The serum levels of estradiol and progesterone were significantly different among the groups, although the endometrial thickness was comparable among the groups. Clinical pregnancy rates were significantly different among the groups. The multivariate logistic regression analysis revealed that the AOR for clinical pregnancy in the OV 4.5 group was comparable to that in the OV 5.0 group (Supplemental Table 2). However, the AOR for clinical pregnancy in the HR group was significantly lower than that in the OV 4.5 group.

### TABLE 1

| Characteristic                        | OV 4.5 | OV 5.0 | HR  | \( P \) value |
|---------------------------------------|--------|--------|-----|--------------|
| Embryo transfer cycles, n             | 29,705 | 31,995 | 5318|              |
| Maternal age, mean±SEM               | 38.2±0.0\(^a\) | 38.6±0.0\(^b\) | 37.9±0.1\(^c\) | <.0001 |
| Body mass index, mean±SEM            | 20.7±0.0\(^a\) | 20.6±0.0\(^b\) | 20.8±0.0\(^b\) | <.0001 |
| Smoking, n (%)                        | 1644 (5.5)\(^a\) | 1577 (4.9)\(^b\) | 157 (3.0)\(^c\) | <.0001 |
| Previous delivery, n (%)             | 2549 (8.6)\(^a\) | 2427 (7.6)\(^b\) | 391 (7.4)\(^b\) | <.0001 |
| Cause of infertility                  |        |        |     |              |
| Ovulation, n (%)                      | 116 (0.4)\(^a\) | 183 (0.6)\(^b\) | 151 (2.8)\(^c\) | <.0001 |
| Tubal factor, n (%)                   | 4169 (14.0)\(^a\) | 4712 (14.7)\(^b\) | 708 (13.3)\(^a\) | .0048 |
| Endometriosis, n (%)                  | 588 (2.0) | 629 (2.0) | 97 (1.8) | .7498 |
| Endometrial factor, n (%)             | 270 (0.9)\(^a\) | 228 (0.7)\(^b\) | 35 (0.7)\(^a\) | .0117 |
| Male factor, n (%)                    | 2985 (10.1)\(^a\) | 3387 (10.6)\(^b\) | 398 (7.5)\(^c\) | <.0001 |
| Combined, n (%)                       | 3849 (13.0)\(^a\) | 3578 (11.2)\(^b\) | 789 (14.8)\(^c\) | <.0001 |
| Unexplained, n (%)                    | 17,728 (59.7) | 19,278 (60.3) | 3140 (59.0) | .1438 |
| Embryo culture time (h), mean±SEM    | 129.3±0.1\(^a\) | 129.8±0.2\(^b\) | 130.0±0.2\(^b\) | <.0001 |
| Blastocyst diameter, mean±SEM        | 183.3±0.1 | 183.1±0.1 | 183.0±0.3 | .3147 |
| Morphologically good blastocysts, n (%)| 10,220 (34.4)\(^a\) | 10,745 (33.6)\(^b\) | 1573 (29.6)\(^c\) | <.0001 |
| Serum estradiol level (pg/mL), mean±SEM | 156.3±0.4\(^a\) | 176.0±0.5\(^b\) | 201.2±1.3\(^c\) | <.0001 |
| Serum progesterone level (ng/mL), mean±SEM | 16.3±0.0\(^a\) | 17.1±0.0\(^b\) | 10.5±0.1\(^c\) | <.0001 |
| Endometrial thickness (mm)            | 10.6±0.1 | 10.4±0.1 | 10.7±0.3 | .3073 |
| Clinical pregnancy, n (%)             | 15,216 (51.2)\(^a\) | 16,016 (50.1)\(^b\) | 2500 (47.0)\(^c\) | <.0001 |
| Singleton pregnancy, n               | 15,095 | 15,903 | 2488 | — |
| Follow-up data on the pregnancy       | 14,950 | 15,722 | 2476 | — |
| Miscarriage, n                        | 5324 | 5752 | 994 | — |
| Deliveries, n                        | 9626 | 9970 | 1482 | — |

HR, hormone replacement; OV, ovulation; SEM, standard error of the mean.

\(^a\)–\(^c\) Different superscript letters indicate a significant difference at \( P < .05 \).

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The maternal outcomes are described in Table 2. No significant differences in maternal outcomes were observed between the OV 4.5 and OV 5.0 group. Significantly higher rates of HDP and placenta accreta were observed in the HR group than in the OV 4.5 and OV 5.0 groups. The incidence of other complications was comparable among the 3 groups. The multivariate logistic regression analysis also revealed a higher AOR for the incidence of HDP and placenta accreta in the HR group (Table 3).

Obstetrical outcomes after single vitrified-warmed blastocyst transfer
No significant differences in obstetrical outcomes were observed between the OV 4.5 and OV 5.0 group (Table 4). Higher rates of cesarean delivery and

### TABLE 2
Pregnancy complications during the perinatal period

| Complication                                      | OV 4.5   | OV 5.0   | HR       | P value  |
|--------------------------------------------------|----------|----------|----------|----------|
| Deliveries, n                                     | 916 (9.5) | 997 (10.0) | 190 (12.8) | .0004    |
| Pregnancy complications, n (%)                   | 287 (3.0) | 331 (3.3)  | 81 (5.5)  | <.0001   |
| Hypertensive disorders of pregnancy, n (%)       | 341 (3.5) | 386 (3.9)  | 62 (4.2)  | .3119    |
| Gestational diabetes mellitus, n (%)             | 14 (0.2)  | 10 (0.1)   | 3 (0.2)   | .4799    |
| HELLP syndrome, n (%)                            | 149 (1.6) | 175 (1.8)  | 24 (1.6)  | .5203    |
| Preterm premature rupture of membranes, n (%)    | 75 (0.8)  | 63 (0.6)   | 8 (0.5)   | .3526    |
| Low-lying placenta, n (%)                        | 14 (0.2)  | 12 (0.1)   | 7 (0.5)   | .0056    |
| Placenta previa, n (%)                           | 29 (0.3)  | 27 (0.3)   | 6 (0.4)   | .6633    |
| Placenta accreta, n (%)                          | 149 (1.6) | 175 (1.8)  | 24 (1.6)  | .5203    |
| Other, n (%)                                      | 30 (0.3)  | 27 (0.3)   | 4 (0.3)   | .8589    |

HELLP, hemolysis, elevated liver enzymes, and low platelet count; HR, hormone replacement; OV, ovulation.

**Confounders:** maternal age, body mass index, smoking, previous delivery, cause of infertility, culture time, and blastocyst morphology.

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### TABLE 3
Logistic regression analysis of pregnancy complications

| Adverse maternal outcomes                        | Group | OR (95% confidence intervals) | P value  | aOR (95% confidence intervals) | P value  |
|--------------------------------------------------|-------|-------------------------------|----------|-------------------------------|----------|
| Pregnancy complicationsa                         | OV 5.0| 1.07 (0.97–1.17)              | .1743    | 1.05 (0.95–1.16)              | .3133    |
|                                                   | HR    | 1.39 (1.17–1.64)              | .0001    | 1.55 (1.30–1.84)              | <.0001   |
| Hypertensive disorders of pregnancya             | OV 5.0| 1.10 (0.94–1.30)              | .2527    | 1.09 (0.92–1.28)              | .2929    |
|                                                   | HR    | 1.84 (1.42–2.38)              | <.0001   | 2.17 (1.67–2.81)              | <.0001   |
| Gestational diabetes mellitusa                   | OV 5.0| 1.10 (0.95–1.28)              | .1786    | 1.09 (0.94–1.28)              | .2210    |
|                                                   | HR    | 1.14 (0.86–0.15)              | .3417    | 1.26 (0.94–1.69)              | .1094    |
| HELLP syndromeb                                  | OV 5.0| 0.64 (0.28–1.43)              | .2782    | 0.66 (0.28–1.51)              | .3270    |
|                                                   | HR    | 1.31 (0.37–4.53)              | .6684    | 1.54 (0.42–5.64)              | .5125    |
| Preterm premature rupture of membranesa          | OV 5.0| 0.92 (0.53–1.60)              | .7855    | 0.91 (0.52–1.58)              | .7428    |
|                                                   | HR    | 1.51 (0.62–3.68)              | .7855    | 1.61 (0.65–3.94)              | .2956    |
| Low-lying placentaa                              | OV 5.0| 0.86 (0.61–1.21)              | .4110    | 0.83 (0.59–1.17)              | .2990    |
|                                                   | HR    | 0.74 (0.35–1.55)              | .4372    | 0.74 (0.35–1.56)              | .4360    |
| Placenta previaa                                 | OV 5.0| 1.15 (0.92–1.43)              | .2111    | 1.12 (0.90–1.41)              | .2914    |
|                                                   | HR    | 1.08 (0.70–1.67)              | .7096    | 1.06 (0.67–1.68)              | .7809    |
| Placenta accretaa                                | OV 5.0| 0.88 (0.40–1.95)              | .7700    | 0.72 (0.33–1.60)              | .4336    |
|                                                   | HR    | 3.54 (1.41–8.89)              | .0071    | 3.85 (1.54–9.60)              | .0038    |
| Placental abruptiona                             | OV 5.0| 0.83 (0.48–1.41)              | .4965    | 0.88 (0.52–1.50)              | .6585    |
|                                                   | HR    | 0.67 (0.20–2.22)              | .5211    | 0.91 (0.32–2.62)              | .8721    |

Reference: OV 4.5 group.

aOR, adjusted odds ratio; HELLP, hemolysis, elevated liver enzymes, and low platelet count; HR, hormone replacement; OR, odds ratio; OV, ovulation.

Confounders: maternal age, body mass index, smoking, previous delivery, cause of infertility, culture time, and blastocyst morphology.

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Preterm delivery were observed in the HR group than in the OV 4.5 and OV 5.0 groups. Shorter birth length and lower birthweight were frequently observed in the HR group compared with the OV 4.5 and OV 5.0 groups. Meanwhile, the incidences of stillbirth, infant death, and birth defects were comparable among the 3 groups. The multivariate logistic regression analysis also demonstrated higher AORs for the incidence of cesarean delivery, preterm delivery, and low birthweight in the HR group (Table 5).

**Detailed analysis of congenital anomalies**

The incidence of each congenital anomaly was similar among the 3 groups (Table 6 and Supplemental Table 3).

### Discussion

#### Principal findings

This study demonstrated that SVBTs in the HR cycle increase the occurrence of HDP, placenta accreta, cesarean delivery, preterm delivery, and low birthweight compared with that in the natural cycle; however, the rate of congenital anomalies did not change. Furthermore, no difference was observed in perinatal outcomes or congenital anomalies because of a half-day difference in the transfer timing in the natural cycle.

#### Results and clinical implications

Given that the risk of SGA did not increase with the increased risk of low birthweight, the latter may have been correlated with the risk of preterm delivery. As for HDP, its risk increased in HR cycles, similarly to what was observed in previous reports. Some studies have discussed the mechanisms by which the risk of HDP increases during the HR cycle: the corpus luteum (CL), which is not formed in HR cycles, produces not only estradiol and progesterone but also vasoactive products such as relaxin, vascular endothelial growth factor, and angiogenic proxies of estrogen, which regulate the maternal circulatory system in early pregnancy. Therefore, the lack of CL-derived ligands might be associated with the increased occurrence of HDP in HR cycles. This highlights the importance of the CL in women with regular ovulatory cycles, noting that endometrial preparation through increased estradiol levels from growing follicles,

| TABLE 4 | Obstetrical outcomes stratified by transfer method |
|---------|---------------------------------------------------|
| Outcomes | OV 4.5 | OV 5.0 | HR | \(P\) value |
| Patients with deliveries, n | 9626 | 9970 | 1482 |
| Completed follow-up data on neonatal outcomes, n (%) | 9388 (97.5) | 9751 (97.8) | 1439 (97.1) | .1705 |
| Patients without cervical incompetence, n | 9375 (99.9) | 9734 (99.8) | 1434 (99.7) | .1990 |
| Live birth, n (%) | 9340 (99.6) | 9701 (99.7) | 1431 (99.8) | .6077 |
| Stillbirth, n (%) | 35 (0.4) | 33 (0.3) | 3 (0.2) | .6077 |
| Cesarean delivery rate, n (%) | 3087 (33.1) \(^a\) | 3219 (33.2) \(^a\) | 621 (43.4) \(^b\) | <.0001 |
| Gestational age, wk, mean±SEM | 39.1±0.0 | 39.1±0.0 | 39.0±0.1 | .2103 |
| ≤27 wk, n (%) | 24 (0.3) | 25 (0.3) | 8 (0.6) | .1128 |
| 28–36 wk, n (%) | 481 (5.2) \(^a\) | 493 (5.1) \(^a\) | 107 (7.5) \(^b\) | .0006 |
| ≥37 wk, n (%) | 8835 (94.6) \(^a\) | 9183 (94.7) \(^a\) | 1316 (92.0) \(^b\) | .0001 |
| Birth length, cm, mean±SEM | 49.0±0.0 \(^a\) | 49.1±0.0 \(^a\) | 48.7±0.1 \(^b\) | <.0001 |
| Birthweight, g, mean±SEM | 3039.2±4.5 | 3033.4±4.3 | 3001±13.1 | .0080 |
| Small for gestational age | 388 (4.2) | 384 (4.0) | 70 (4.9) | .2431 |
| Large for gestational age | 1569 (16.8) | 1528 (15.8) | 237 (16.6) | .1410 |
| Infant sex | | | | |
| Male, n (%) | 4805 (51.5) | 5055 (52.1) | 783 (54.7) | .0663 |
| Female, n (%) | 4535 (48.5) | 4646 (47.9) | 648 (45.3) | |
| Infant death, n (%) | 8 (0.1) | 12 (0.1) | 3 (0.2) | .3845 |
| Birth defect, n (%) | 230 (2.5) | 247 (2.6) | 38 (2.7) | .8788 |
| Stillbirth | | | | |
| Birth defect, n (%) | 4 (11.4) | 3 (9.1) | 1 (33.3) | .4452 |

\(^{HR}\), hormone replacement; \(^{OV}\), ovulation; \(^{SEM}\), standard error of the mean.

\(^{a,b}\) Different superscript letters indicate a significant difference at \(P<.05\).

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natural LH surges, and CL development in the natural cycle is the preferred approach.12

In this study, the risk of placenta accreta was higher in HR cycles than in natural cycles. The reported prevalence of placenta accreta is 0.17%31; in this study, it was 3 times higher in HR cycles. Placenta accreta is caused by trophoblast penetration into the myometrium or direct contact between the myometrium and chorionic villi owing to defective formation of the decidua, with a history of uterine surgery being a risk factor.32 Unfortunately, history of uterine surgery was not included in the survey and was not analyzed in this study.

Strengths and limitations
The strength of this study was its analysis of a large dataset from a single center. In addition, the use of drugs for endometrial preparation, techniques of oocyte retrieval and transfer, and culture conditions were uniform. Therefore, potential bias owing to differences

### TABLE 5
Logistic regression analysis of neonatal outcomes

| Adverse neonatal outcomes | Group | OR (95% confidence intervals) | P value | aOR (95% confidence intervals) | P value |
|---------------------------|-------|-----------------------------|---------|-------------------------------|---------|
| Stillbirth<sup>a</sup>    | OV 5.0 | 0.90 (0.56–1.46)            | .6906   | 0.90 (0.57–1.52)              | .8074   |
|                           | HR    | 0.55 (0.17–1.82)            | .3349   | 0.64 (0.19–2.10)              | .4569   |
| Cesarean delivery<sup>a</sup> | OV 5.0 | 1.00 (0.94–1.06)            | .8460   | 1.00 (0.94–1.06)              | .9516   |
|                           | HR    | 1.55 (1.38–1.73)            | <.0001  | 1.94 (1.71–2.19)              | <.0001  |
| Preterm delivery (<37 wk)<sup>a</sup> | OV 5.0 | 0.98 (0.87–1.11)            | .8371   | 0.97 (0.85–1.10)              | .6667   |
|                           | HR    | 1.52 (1.23–1.88)            | <.0001  | 1.54 (1.24–1.92)              | <.0001  |
| Low birthweight (<2500 g)<sup>a</sup> | OV 5.0 | 1.01 (0.90–1.12)            | .8334   | 1.00 (0.90–1.11)              | .9356   |
|                           | HR    | 1.36 (1.13–1.64)            | .0010   | 1.43 (1.18–1.73)              | .0003   |
| Small for gestational age<sup>a</sup> | OV 5.0 | 0.95 (0.82–1.09)            | .4935   | 0.95 (0.82–1.10)              | .4970   |
|                           | HR    | 1.18 (0.91–1.54)            | .1984   | 1.21 (0.92–1.58)              | .1591   |
| Large for gestational age<sup>a</sup> | OV 5.0 | 0.95 (0.85–1.05)            | .1502   | 0.92 (0.85–1.00)              | .0532   |
|                           | HR    | 0.98 (0.84–1.14)            | .8233   | 0.99 (0.84–1.15)              | .9020   |
| Infant death<sup>a</sup> | OV 5.0 | 1.44 (0.59–3.53)            | .4204   | 1.38 (0.56–3.39)              | .4752   |
|                           | HR    | 2.45 (0.64–9.24)            | .1859   | 2.53 (0.66–9.65)              | .1731   |
| Birth defect<sup>a</sup> | OV 5.0 | 1.01 (0.86–1.19)            | .8797   | 1.00 (0.83–1.21)              | .9431   |
|                           | HR    | 1.14 (0.84–1.55)            | .3803   | 1.17 (0.82–1.66)              | .3703   |

Reference: OV 4.5 group.

aOR, adjusted odds ratio; HR, hormone replacement; OR, odds ratio; OV, ovulation.

<sup>a</sup> Confounders: maternal age, body mass index, smoking, previous delivery, cause of infertility, culture time, blastocyst morphology, and infant sex.

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### TABLE 6
Congenital anomalies stratified by transfer method

| Congenital anomalies | OV 4.5 | OV 5.0 | HR  | P value |
|----------------------|--------|--------|-----|---------|
| Live birth, n        | 9340   | 9701   | 1431|         |
| Nervous system, n (%)| 17 (0.2)| 12 (0.1)| 2 (0.1)| .5851   |
| Congenital heart defects, n (%) | 114 (1.2) | 111 (1.1) | 22 (1.5) | .4392 |
| Respiratory, n (%)   | 2 (0.0) | 4 (0.0) | 0 (0) | .5800   |
| Orofacial clefts, n (%) | 9 (0.1) | 12 (0.1) | 2 (0.1) | .8104   |
| Digestive systems, n (%) | 9 (0.1) | 15 (0.2) | 1 (0.1) | .4337   |
| Abdominal defects, n (%) | 2 (0.0) | 1 (0.0) | 0 (0) | .7325   |
| Urinary, n (%)       | 26 (0.3) | 29 (0.3) | 3 (0.2) | .8356   |
| Genital, n (%)       | 4 (0.0) | 3 (0.0) | 2 (0.1) | .0842   |
| Limb, n (%)          | 16 (0.2) | 17 (0.2) | 5 (0.4) | .3276   |
| Other congenital abnormalities, n (%) | 4 (0.0) | 2 (0.0) | 0 (0) | .5366   |
| Chromosomal, n (%)  | 33 (0.4) | 49 (0.5) | 2 (0.1) | .0850   |
| Stillbirth, n        | 35     | 33     | 3    |         |
| Congenital heart defects, n (%) | 1 (2.9) | 0 (0) | 0 (0) | .5936   |
| Orofacial clefts, n (%) | 0 (0) | 0 (0) | 1 (33.3) | .0789 |
| Limb, n (%)          | 1 (2.9) | 0 (0) | 0 (0) | .5936   |
| Chromosomal, n (%)  | 2 (5.7) | 3 (8.1) | 0 (0) | .7660   |

HR, hormone replacement; OV, ovulation.

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in the detailed conditions that potentially occur in multicenter data collection is not likely.

This study also had certain limitations. First, the patient background was not uniform. When transferring a thawed blastocyst, endometrial preparation is performed in the natural cycle, and an HR cycle is selected when endometrial adjustment fails or is expected to fail in the ovulatory cycle because of ovulation failure or DOR. Therefore, differences in patient backgrounds that cannot be adjusted from these data may be biased. One of the several reasons for selecting endometrial adjustment in this study may be DOR considering the patient backgrounds. As to whether DOR worsens perinatal outcomes, a previous study reported no significant difference in perinatal outcomes compared with normal responders, whereas another study revealed that HDP is more common in DOR; nevertheless, no consensus has been reached. Further studies are necessary to reveal the influence of ovarian function and reserve on perinatal outcomes. Furthermore, patient preferences and schedules were also considered when the endometrial preparation was decided. Most patients desire to minimalize the use of exogenous hormones to reduce economic and physical burden. Meanwhile, some patients prioritize their schedule and select the HR cycle. These differences in patient preference might have led to bias in this study. Second, this study lacked data on the number of previous ART cycles. Furthermore, data on maternal and perinatal outcomes were collected using self-reported parental questionnaires. Self-reported maternal and perinatal complications could be potentially erroneous, particularly where uncommon or complex medical terms were involved. Thus, a crosschecking process could have made the data more credible. Moreover, buserelin was used for ovulation trigger in this study; the results might vary when human chorionic gonadotropin is used as the trigger. Therefore, to validate our findings, a comparative study between the HR cycle and natural cycle with human chorionic gonadotropin is required.

Conclusions

The risk of HDP, placenta accreta, cesarean delivery, preterm delivery, and low birthweight was higher in HR cycles than in natural cycles, whereas the risk of congenital anomalies was similar between the 2 types of cycles. Furthermore, no difference was observed in perinatal outcomes or congenital anomalies because of a half-day difference in transfer timing in the natural cycle. As frozen-thawed transfers become the mainstream method of ART, the HR method of endometrial preparation will become increasingly convenient given that it allows easy schedule adjustment. However, further follow-up is needed to investigate these risks and explore improved endometrial preparation methods.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.xagr.2022.100081.

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