Title: Natural cycles in frozen-thawed embryo transfer are associated with lower risks of preeclampsia and large-for-gestational-age infants than artificial cycles: A systematic review and meta-analysis

Running title: Perinatal outcomes after natural and artificial cycles

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Abstract

This systematic review of literature and meta-analysis of observational studies that reported on perinatal outcomes after FET aims to determine whether in singleton pregnancies conceived after in vitro fertilization (IVF), do natural cycle frozen embryo transfer (NC-FET) decrease the risk of adverse perinatal outcomes compared to artificial cycle frozen embryo transfer (AC-FET). Thirteen cohort studies, including 93201 cycles, matched the inclusion criteria. NC-FET is associated with a lower risk of hypertensive disorders in pregnancy (HDP) (RR 0.61; 0.50-0.73), preeclampsia (RR 0.47; 0.42-0.53), large for gestational age (LGA) (RR 0.93; 0.90-0.96) and macrosomia (RR 0.83; 0.70-0.99) compared to AC-FET. There was no significant difference in the risk of gestational hypertension. Regarding the secondary outcomes, the risk of preterm birth, post-term birth, low birth weight, small for gestational age, cesarean section, postpartum haemorrhage, placental abruption and placenta accreta were significantly lower with NC-FET than with AC-FET. Concerning safety, NC-FET significantly decreases the risk of HDP, preeclampsia, LGA, macrosomia, preterm birth, post-term birth, low birth weight, small for gestational age, cesarean section, postpartum hemorrhage, placental abruption and placenta accreta. Further randomized controlled trials that address the effect of NC-FET and AC-FET on maternal and perinatal outcomes are warranted.

Key words: frozen-thawed embryo transfer / natural cycle / hypertensive disorders of pregnancy / preeclampsia / macrosomia /

Introduction

Frozen-thawed embryo transfer (FET) has increased steadily over the past few years worldwide (De Geyter et al., 2020; Zegers-Hochschild et al., 2020). In 2015, FET accounted for approximately 40% of all in vitro fertilization (IVF) cycles in Europe (De Geyter et al., 2020).

The freeze-all strategy has reported successful results with higher live birth rates and lower incidence of ovarian hyperstimulation syndrome compared to fresh embryo transfer (ET) (Roque et al., 2019; Wei et al., 2019). Some studies suggest that singletons born after FET also have better neonatal outcomes compared with singletons born after fresh ET regarding low birth weight (LBW), small for gestational age (SGA), and preterm birth (PTB) (Roque et al., 2019; Wei et al., 2019; Zhang et al., 2018).

However, compared with fresh ET, FET seems to carry a greater risk of hypertensive disorders in pregnancy (HDP), including gestational hypertension and preeclampsia (Opdahl et al., 2015; Roque et al., 2019), being born as large for gestational age (LGA) and macrosomia (Pinborg et al., 2014; Rodriguez-Wallberg et al., 2019; Wei et al.,2019). The reasons behind these findings are not clearly understood, but it was suggested that some cryoprotectants or the vitrification and thawing process per se could develop some metabolic or epigenetic changes related to abnormal placentation and eventually preeclampsia (Hiura et al., 2017; Nelissen et al., 2011).

The role of the endometrium has also been a focus of attention. There are different options described to prepare the endometrium for FET, including a natural cycle (NC-FET) based on the detection of the endogenous Luteinizing hormone (LH) surge in the blood, a modified natural cycle using human Chorion Gonadotropin (hCG) for final oocyte maturation, and artificial cycle (AC-FET) based on an hormonal replacement treatment with or without co-treatment with a Gonadotropin releasing hormone (GnRH) analog, and a stimulated cycle with antiestrogens, aromatase inhibitors or gonadotropins (Lawrenz et al., 2020). The most commonly used type of endometrial preparation is the AC-FET (Lawrenz et al., 2020), in which the administration of estrogens trying to mimic the changes generated by steroids of ovarian origin on the natural cycle. Endometrial thickness and pattern are monitored by vaginal ultrasound and progesterone

administration is usually started from an endometrial thickness of approximately 7–8 mm (Liu et al., 2018). In clinical practice, AC-FET is popular because it involves less monitoring and the ET can be scheduled on a convenient day for the patient and the practice. (Singh et al., 2020).

In the last years, multiple studies have studied the effectiveness of different endometrial preparation schemes regarding implantation rates, clinical pregnancy rates or live birth rates (Yarali et al., 2016; Ghobara et al., 2017; Mackens et al., 2017; Groenewoud et al., 2018). However, studies evaluating the perinatal and maternal outcomes are scarce and reported dissimilar results. While some observational data have suggested higher rates of HDP, LGA and macrosomia after programmed FET cycles compared with NC-FET (Ginstrom Ernstad et al., 2019; Saito et al., 2019; Wang et al., 2020; Zong et al., 2020), other studies have not confirmed these findings (Lin et al., 2020; Pan et al., 2020; Wang et al., 2020b).

The reason behind the high rates of these adverse obstetrical outcomes is not known, but it has been suggested that some endometrial changes mediated by the altered levels of estradiol and progesterone possibly reached during the AC-FET, could be associated to the development of an impaired decidualization and placentation, leading to placenta-related complications such as HDP, placenta accreta, placenta previa and placental abruption (Chen et al., 2012; Kaser et al., 2015; Schatz et al., 2016).

In addition, the hypothalamic pituitary-gonadal axis is inhibited by exogenous estradiol. This can lead to a lack of a corpus luteum (CL). The CL is the primary producer of hormones including reproductive ones, for example, relaxin. Recently published studies found that scheduled FET cycles, where no CL is present, were associated with higher rates of preeclampsia compared with

natural FET cycles, where one or more CL occur (Conrad et al., 2013; von Versen-Höynck et al., 2019).

Given the increasing use of FET, there is a critical need to determine if specific FET protocols could be related to the development of adverse obstetric and maternal outcomes; and if elements of the treatment could be modified to optimize outcomes. Therefore, we aim to determine whether NC-FET decreases the risk of adverse perinatal outcomes compared to AC-FET.

Methods

Search strategy and selection criteria

We conducted a systematic review and meta-analysis based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Shamseer et al., 2015). The study protocol is accessible at https://inplasy.com/ (registration number INPLASY202060088).

The selection criteria were described according to Patients, Intervention, Comparison and Outcomes (PICO) statements. Studies that compared the reproductive outcomes between NC-FET and AC-FET were included (Table I).

An electronic search was performed using databases, including PubMed, SCOPUS and the Cochrane database, from 1982 through March 2020. We also searched the reference lists of relevant articles for any additional studies not covered by the literature search. The search combined terms and descriptors related to variants for the interventions, population study and outcomes: IVF with or without intracytoplasmic sperm injection (ICSI), frozen-thawed embryo transfer, endometrial preparation, natural cycle, hormone replacement cycle, hypertensive disorders in pregnancy, preeclampsia, large for gestational age, macrosomia, preterm birth, post-term birth, low birth weight, very low birth weight, small for gestational age, stillbirth, neonatal

mortality, gestational diabetes, cesarean section, placenta previa, placenta accreta, placental abruption and postpartum hemorrhage. The search strategy was modified to fit with the syntaxes used in each database consulted.

Study selection and data extraction

All of the abstracts retrieved from the search in a first screening were assessed by two researchers (JM and MC), independently. Next, full texts of citations that fit the inclusion criteria were screened. Both authors judged study eligibility, assessed quality, and extracted data solving discrepancies by agreement, and if required, reaching a consensus with a third author (JE). Data extracted was summarized for each outcome (Tables II and III). The authors referred to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) to evaluate the quality of evidence for each outcome (Schünemann et al., 2018).

Risk of Bias assessment

Study quality was assigned by two reviewers (JM and MC) following the guidelines described in the Newcastle-Ottawa Scales (NOS) for assessing the quality of included studies (Stang et al., 2010). Assessment of bias was performed on the studies regarding the selection process, comparability of cohorts and outcomes ascertainment. The quality assessment and risk of bias of the included studies are presented in Table I.

Outcome Measures

The primary outcome measure was the rate of adverse pregnancy outcomes including:

Hypertensive disorders in pregnancy (HDP), defined as a blood pressure of ≥140 / 90 mmHg on two or more occasions, at least 6 hours apart, and more than 20 weeks of gestation; gestational hypertension, defined as hypertension arising de novo after 20 weeks' gestation in the absence of proteinuria and without biochemical or hematological abnormalities; preeclampsia, diagnosed by the presence of de novo hypertension after 20 weeks' gestation accompanied by proteinuria and/or evidence of maternal acute kidney injury, liver dysfunction, neurological features, hemolysis or thrombocytopenia, and/or fetal growth restriction (Brown et al., 2018; ACOG., 2019); large for gestational age (LGA), defined as birth weight above the 90th percentile; and macrosomia defined as birth weight above 4000 g.

Secondary outcomes measures were preterm birth (PTB) defined as a live birth before 37 weeks; post-term birth defined as a live birth after 42 weeks; low birth weight (LBW) defined as a birth weight below 2500 g; very low birth weight (VLBW) defined as a birth weight below 1500 g; small for gestational age (SGA), defined in two ways: Birth weight under 2 standard deviations or below the 10th percentile; stillbirth defined as the death of a fetus prior to the complete expulsion or extraction from its mother after 22 completed weeks of gestational age; neonatal mortality defined as death before 28 days postpartum; gestational diabetes, cesarean section, placenta previa, placenta accreta, placental abruption and postpartum hemorrhage.

The definitions that have been used were in line with the ones prescribed by the International Society for the Study of Hypertension in Pregnancy (ISSHP) (Brown et al., 2018), The American

College of Obstetricians and Gynaecologists (ACOG., 2019), The International Committee Monitoring Assisted Reproductive Technologies/World Health Organization glossary (Zegers-Hochschild et al., 2017) and according to ICD-10 codes gathered from the maternal hospital discharge data in the different articles included in this study.

Quantitative analysis

In order to ascertain the pooled impact of different variables, we made use of a Mantel-Haenszel model and utilized the fixed-effects model. We calculated the risk ratio (RR) for dichotomous data along with the 95% confidence intervals (CIs). We assessed the extent of dissimilarity between studies attributable to heterogeneity with the I square statistics (I^2). We used the random-effects model (Higgins et al., 2003) in cases where the heterogeneity was greater than 50% (I^2 >50%). The Review Manager (RevMan Version 5.3 Software, Copenhagen, Denmark) was utilized for statistical analysis.

Results

The search yielded a total of 1903 records but 1868 were excluded after we screened the titles and abstracts of these manuscripts. Out of the remaining 35 studies that were considered eligible by one or both reviewers, only 13 were included in the present systematic review and meta-analysis. Figure 1 represents in detail the study selection process.

Description of Included Studies

Thirteen studies investigating perinatal and maternal outcomes in pregnancies after NC-FET versus AC-FET met the inclusion criteria. Details of the included studies were summarized in Table I. All of the studies had NOS scores >7 were considered to be of high quality.

Synthesis of results

Natural cycles vs artificial cycles in primary outcomes

Hypertensive disorders in pregnancy

Ten studies reported HDP including 26661 patients in the NC-FET group and 23558 patients in the AC-FET group. The overall RR for HDP was 0.61 (95% CI: 0.50–0.73; I2 = 65%; Fig. 2a), favoring the NC-FET group. The quality of evidence was low according to GRADE. We performed a sub-analysis considering preeclampsia or gestational hypertension separately. Women who had their delivery after NC-FET were at an decreased risk of preeclampsia compared with those after AC-FET (four studies, RR = 0.47; 95% CI: 0.42–0.53; I2 = 4%; low quality of evidence; Fig. 2b). No differences were noted between the NC-FET and AC-FET groups regarding the risk of gestational hypertension (three studies, RR = 0.72; 95% CI: 0.51–1.02; I2 = 0%, low quality of evidence; Fig. 2c).

Large for gestational age

There were nine studies, including 77629 patients, related to the complication of LGA. A lower risk of LGA (RR = 0.93; 95% CI:0.90–0.96; I2 = 0%) was found in pregnancies after NC-FET cycles than after AC-FET (low quality of evidence; Fig. 3a).

Macrosomia

Ten studies, including 72886 patients, provided the outcome of macrosomia. A lower risk of macrosomia was found in pregnancies after NC-FET cycles than after AC-FET cycles (RR = 0.83; 95% CI: 0.70–0.99; I2 = 70%; low quality of evidence; Fig. 3b).

Natural cycles vs artificial cycles in secondary outcomes

Preterm birth

Twelve studies were involved in the meta-analysis, including 78584 patients. Analysis showed that the overall risk of PTB was significantly lower among pregnancies resulting from the NC-FET cycles (RR = 0.83; 95% CI: 0.79–0.88; I2 = 39%; low quality of evidence; Supplementary Fig. S1a).

Post-term birth

Seven studies, which included 67262 patients, reported results comparing the post-term rates for each group. The overall RR for post-term birth was 0.48 (95% CI: 0.29–0.80; I2 = 81%; very low quality of evidence; Supplementary Fig. S1b) favoring the NC-FET group.

Low birth weight

Ten studies reported the prevalence of LBW, which included 78656 patients. The RR and the 95% CI of having LBW were 0.84 and 0.80–0.89 after NC-FET compared with AC-FET (I2 = 13%; low quality of evidence; Supplementary Fig. S1c).

Very low birth weight

Five studies, including 29453 patients, were analyzed. The overall risk of VLBW was not significantly different among pregnancies resulting from the NC-FET and AC-FET cycles (RR = 0.91; 95% CI: 0.66–1.26; I2 = 4%; low quality of evidence; Supplementary Fig. S1d).

Small for gestational age

Nine studies that included 77629 patients reported SGA data. The overall risk of SGA observed after NC-FET compared with AC-FET was not significantly different (RR = 0.94; 95% CI:0.88–1.00). There was minor heterogeneity among the studies (I2 = 0%; low quality of evidence; Supplementary Fig. S2a).

Stillbirth

Seven studies, including 33890 patients, were used in this analysis. The overall risk of stillbirth was not significantly different among the pregnancies resulting from the NC-FET and AC-FET cycles (RR = 0.86; 95% CI: 0.60–1.24; I2 = 49%; very low quality of evidence; Supplementary Fig. S2b).

Neonatal mortality

Three studies, including 11931 patients, were analyzed. Overall, no difference in the risk of neonatal mortality was noted among pregnancies resulting from NC-FET and AC-FET cycles (RR = 0.55; 95% CI: 0.22–1.37; I2 = 0%; very low quality of evidence; Supplementary Fig. S2c).

Birth defects

Four studies, including 9501 patients, reported birth defects data. The risk of birth defects was not significantly different in pregnancies resulting from the NC-FET than from AC-FET (RR = 0.87; 95% CI: 0.68–1.12; I2 =0%; very low quality of evidence; Supplementary Fig. S2d).

Cesarean section

Eight studies, including 70404 patients, were pooled in this analysis. A significantly lower risk in cesarean section was observed after NC-FET group compared with the AC-FET group (RR = 0.83; 95% CI: 0.77–0.90; I2 =95%; very low quality of evidence; Supplementary Fig. S3a) but heterogeneity was substantial.

Postpartum hemorrhage

Four studies, including 23343 patients, were involved in the meta-analysis. A significant difference in the rate of postpartum hemorrhage was noted among the offspring resulting from the NC-FET and AC-FET strategies (RR = 0.39; 95% CI: 0.35–0.45; I2 =30%; low quality of evidence; Supplementary Fig. S3b).

Gestational diabetes

Ten studies, including 57012 patients, were analyzed. The overall risk of gestational diabetes was not significantly different among pregnancies resulting from the NC-FET and AC-FET cycles (RR = 1.03; 95% CI: 0.77–1.38). There was marked heterogeneity among the studies (I2 = 90%; very low quality of evidence; Supplementary Fig. S3c).

Placental abruption

Five studies, which included 47370 patients, were involved in the meta-analysis. Analysis showed that the overall risk of placental abruption was significantly lower among pregnancies resulting from the NC-FET cycles (RR = 0.61; 95% CI: 0.38–0.98; I2 = 0%; low quality of evidence; Supplementary Fig. S3d).

Placenta previa

Eight studies, including 57740 patients, evaluated the placenta previa rates. No difference was noted in the placenta previa rates between NC-FET and AC-FET cycles (RR = 0.84; 95% CI: 0.60–1.19; I2 = 64%; low quality of evidence; Supplementary Fig. S3e).

Placenta accreta

Three studies that included 36139 patients were pooled in this meta-analysis. Overall, a statistical difference was noted in the placenta accreta rates between the NC-FET and AC-FET cycles (RR = 0.19; 95% CI:0.11–0.33; I2 = 0%; low quality of evidence; Supplementary Fig. S3f).

Sensitivity analysis

Sensitivity analyses were performed to examine the influence of variation among studies on the overall risk estimates. No significant impact was noted on the pooled effect size (Supplementary Data)**.**

Discussion

Main Results

This systematic review and meta-analysis shows a decrease in the risk of HDP, preeclampsia, LGA and macrosomia with the use of NC-FET in preference to AC-FET in the overall population undergoing FET. Low-quality evidence also shows that the use of NC-FET does not lead to any differences in the chances of gestational hypertension.

The GRADE quality of evidence was low mainly because this is a review based on observational studies and due to the substantial inter-study heterogeneity obtained, that was assumed to be created by the variation between study populations.

Strengths

The large sample size is a major strength of this study; the selected articles included two population-based national registries from Sweden and Japan. This large sample allowed investigating relatively infrequent events, like minor obstetrical complications.

We considered studies that utilized suitable research designs that matched for potential confounders such as multiple births and maternal age. Furthermore, the meta-analysis indicates acceptable values through low I^2 values and narrow confidence levels for primary outcomes such as preeclampsia, gestational hypertension, LGA, and secondary outcomes such as PTB, LBW, postpartum hemorrhage, placental abruption and placenta accreta. This implies that the precision of the meta-analysis is of good quality and that the estimated value is comparatively stable for these variables. This systematic review and meta-analysis were performed in accordance with the PRISMA statement. This ensured that the methodological quality was high. Moreover, the

quality of evidence was rated according to GRADE. The validity of our results is notably improved due to these factors.

Limitations

All articles in this review comprised observational studies. A limitation of register-based studies is the inherent lack of data. Hence, we were unable to adjust for potential confounding variables such as parity, smoking status, alcohol intake, socioeconomic status, duration of infertility, women's ovulatory status, body mass index, embryo quality, previous cesarean section, the freezing protocol, the embryo stage of development for transfer and the preimplantation genetic testing. The reason for the use of AC-FET deserves attention because it may be associated with a higher risk of perinatal complications, possibly distorting the outcomes of the analyses. In this study, this point could not be analyzed. In addition, unpublished data as full-text articles and in languages other than English were excluded from the meta-analysis.

Comparison to Other Studies

Our review is consistent with the latest reviews published regarding a lower risk of HDP and preeclampsia by NC-FET patients compared with AC-FET.

A population-based registry study in Sweden including all IVF singleton deliveries from autologous oocytes during the years 2005 - 2015 grouped into FET in programmed, stimulated, or natural cycles and fresh ET (Ginstrom Ernstad et al., 2019). Compared to NC-FET, AC-FET cycles were associated with a higher risk of hypertensive disorders in pregnancy (aOR, 1.78; 95% CI, 1.43-2.21) and postpartum hemorrhage (aOR, 2.63; 95% CI, 2.20-3.13). These results are also in accordance with a recent study suggesting an increased rate of preeclampsia in

programmed FET cycles where no CL is present (von Versen-Höynck et al., 2019a). In the same study, no differences were observed regarding the risk of gestational hypertension comparing programmed FET, natural FET, fresh ET cycles and spontaneous conception.

A retrospective cohort study of patients who conceived after AC-FET and those who conceived after NC-FET was performed based on the Japanese assisted reproductive technology registry in 2014. Multiple logistic regression analyses were performed to investigate potential confounding factors. They reported that pregnancies after AC-FET had increased odds of HDP (aOR, 1.43; 95% CI 1.14–1.80) and placenta accreta (aOR, 6.91; 95% CI, 2.87–16.66) in comparison to pregnancies after NC-FET (Saito et al., 2019).

Regarding LGA, the same authors and other studies (Jing et al., 2019; Pan et al., 2020; Saito et al., 2017; Wang et al., 2020a) did not find similar results to ours, reporting no statistical differences in the risk of LGA for the AC-FET group. Including new studies (Wang et al., 2020b; Zong et al., 2020), our findings show a lower risk for LGA in the NC-FET group.

Regarding macrosomia, the two pieces of research talked about earlier found a significantly higher risk of macrosomia in the AC-FET group (Ginstrom Ernstad et al., 2019; Saito et al.,2019). Ginstrom Ernstad et al reported that a higher risk of macrosomia was seen following programmed FET with an aOR of 1.62 (CI 95% 1.26 - 2.09). These findings are in accordance with our results, but heterogeneity was substantial.

Our research is coherent with other investigations concerning the risk for cesarean section (Ginstrom Ernstad et al., 2019; Saito et al., 2017; Saito et al.,2019; Vidal et al., 2017), preterm birth (Saito et al., 2019; Wang et al., 2020a; Wang et al., 2020b; Zong et al., 2020), post-term birth (Ginstrom Ernstad et al., 2019; Guan et al., 2016, Saito et al., 2017; Saito et al., 2019), LBW (Saito et al., 2017; Saito et al., 2019; Zong et al., 2020), postpartum hemorrhage (Ginstrom

Ernstad et al., 2019; Wang et al., 2020a) and placenta accreta (Saito et al., 2019) among pregnancies after FET.

Saito et al (2019) found an elevated risk of gestational diabetes in AC-FET patients; however, significant differences did not appear in our review. A possible cause for this could be that the meta-analysis included different recent studies which expanded the number of cases. However, one must assess these results cautiously, keeping in mind possible variations between how this condition has been defined by the different studies included (Guan et al., 2016; Jing et al., 2019).

Interpretation of the Results

HDP, preeclampsia and placental disease

Our results indicate a possible link between endometrial preparation and adverse perinatal and maternal outcomes, mainly placenta-related diseases. The success of pregnancy is dependent on proper implantation and placentation. If any problem arises during this process, the production of vasculogenic and angiogenic factors could be affected, leading to changes that are linked with the appearance of major placental syndromes (Morgan., 2016; Thilaganathan., 2017).

The term “placental insufficiency” refers to the atypical movement of uteroplacental nutrients, that can cause damage to the placenta as well as known pregnancy complications such as intrauterine fetal growth restriction and preeclampsia. The severity of placental disease could influence the common underlying etiology for many cases of preeclampsia and PTB (Dude et al., 2017; Morgan., 2016).

The adverse perinatal and maternal outcomes associated with cryopreservation might be originated by supraphysiological steroid hormones levels during early trophoblast invasion, which lead to an anomalous placental development (Choux et al., 2019; Hiura et al., 2017; Lyall

et al., 2013). The AC-FET might be less 'physiological' than a NC-FET cycle due to the preparation of the endometrium with hormonal replacement requiring medication (Groenewoud et al., 2013). In the physiologic implantation process, progestin is important in the decidualization of estradiol-primed human endometrial stromal cells, as the extravillous trophoblast (EVT) invasion and vascular remodeling (Schatz et al., 2016). Uterine spiral arteries and arterioles are converted to low resistance, high capacity vessels that deliver increased blood flow required by the developing fetal-placental unit. A shallow EVT invasion and impaired spiral artery remodeling are linked to preeclampsia, placental abruption, stillbirth, fetal growth restriction and many cases of spontaneous PTB (Chen et al., 2012; Labarrere et al., 2017; Schatz et al., 2016).

Sex steroids are critical modulators of a wide range of maternal and placental process during pregnancy; regulating the uteroplacental vasculature (Maliqueo et al., 2016). Exogen hormonal supplementation leads to increased estrogen and progesterone milieu during implantation and early pregnancy. Even after hormone supplementation is discontinued and ovarian hormone production declines, estradiol and progesterone levels remain persistently elevated in mothers of pregnancies conceived with IVF compared to those who conceived spontaneously, at a time when the placenta becomes the source of hormone production (Lee et al., 2017). Metabolomics studies suggest that the supraphysiologic hormonal state during hormone supplementation may produce a reprogrammation of the trophoblast post-implantation, leading to a dysfunctional hormone production from syncytiotrophoblasts (Sun et al., 2019). Previous studies in animals assessed the impact of elevated estradiol levels in the first trimester, reporting decreased extravillous trophoblast invasion of the uterine spiral arteries (Albrecht et al., 2006). Recent studies have suggested that the impairment in placentation can occur via estradiol-induced

differential expression of the GATA3 transcription factor (Lee et al., 2016) and the Grb10 gene (Mainigi et al., 2016). Elevated progesterone, like elevated estradiol, also impacts placentation which in conjunction with estrogen induces first-trimester trophoblast tubulogenesis through the lysophosphatidic acid pathway (Beltrame et al., 2018). As the elevation in steroids leads to dysfunction in trophoblast cells, these increased hormonal states may be contributors to adverse obstetric and perinatal outcomes, including low birth weight, fetal growth restriction, preeclampsia, and abnormal placentation (Beltrame et al., 2018; Lee et al., 2016; Mainigi et al., 2016). Further studies are necessary to determine the exact mechanisms leading to these outcomes.

On the other hand, corpus luteum has become the focus of new research related to this topic. There is a link between the absence of a corpus luteum due to the pituitary-ovarian axis suppression by estradiol replacement in the context of a programmed cycle and the absence of products of the corpus luteum that are not administered, such as relaxin. This hormone has a significant role for maternal cardiovascular adaptation to pregnancy (Conrad et al., 2013; 2019b). Deficient circulatory adaptations during the first trimester in women conceiving after AC-FET (with the absence of the CL), is also linked to adverse pregnancy outcomes, including preeclampsia (Conrad et al., 2013; 2019a; 2019b; von Versen-Höynck et al., 2019a; 2019b). FET with a natural cycle does not have hormonal substitution and enables the more physiological development of a corpus luteum. The results from a current randomized trial where 75% of the FETs were performed in a natural, ovulatory cycle indicated no increased risk of preeclampsia compared with those with fresh ET (Shi et al., 2018). Results from another study stated that there was a link between programmed FET cycles and higher rates of preeclampsia (12.8% versus

3.9%; P = 0.02) and preeclampsia with severe features (9.6% versus 0.8%; P = 0.002) in contrast to modified natural FET cycles (Conrad et al., 2013).

Birthweight

The causes of increased risk of high birth weight, LGA and macrosomia after FET are still unknown. Epigenetic disturbances during the early embryonic stages as a result of the freezing and thawing procedures might affect the developmental programming of fetal and placental tissues in FET offspring (Pinborg et al., 2016). There might be asynchrony between the embryo and the endometrium in FET cycles that influence fetal growth and development resulting in increased birth weight (Berntsen et al., 2018; Pinborg et al., 2016). Two studies found that placentas after FET were larger than after fresh ET (Choux et al., 2019; Rizzo et al., 2016). Increased placental size has been suggested to be associated with fetal hyperglycemia and indirectly to fetal overgrowth (Acosta et al., 2015) and could explain the increased risk of LGA and macrosomia in FET offspring. In addition, a higher birth weight could be the result of epigenetic changes after IVF with altered methylation of genes involved in the metabolism of insulin growth factor 1 and 2 (Miles et al., 2007).

In the NC-FET group, the intrauterine environment may be more favorable to embryo growth because it is not affected by hormonal replacement in comparison with AC-FET. As mentioned above, higher levels of sex steroids could negatively affect the peri-implantation uterine environment. One study reported an association between excessive fetal growth and preeclampsia, especially late-onset (Rasmussen et al., 2003). Both adverse outcomes may all be related to alterations in the implantation and early fetal developmental stages and need to be explored further.

Secondary Outcomes

The cesarean section rate observed in this review was 41.3%, while other studies reported rates from 28 to 85.9% (Ginstrom Ernstad et al., 2019; Jing et al.; 2019). It should also be noted that such a high cesarean section rate is also associated with other risks, mainly maternal risks such as postpartum hemorrhage. The endometrial preparation using AC-FET itself or pregnancy complications may be responsible for the higher rate of cesarean section in AC-FET patients compared to NC-FET. In addition, socio-cultural differences between the population of the different studies included in the meta-analysis could influence the cesarean section rates (i.e. asian versus european population). Since adverse maternal outcomes, such as HDP, are a significant risk factor for cesarean section, adjusting for confounding factors including the incidence of hypertension is required in future studies in order to clarify whether the higher incidence of cesarean section may be caused by the AC-FET itself.

In this review, we also observed a lower risk of placenta accreta in the NC-FET group. Kaser et al (2015) reported that FET constitutes an independent risk factor for placenta accreta, after controlling for patient age, prior cesarean section, placenta previa and uterine factor infertility. This risk may be directly related to factors associated with cryopreservation, including the freeze-thawing process itself and the mode of uterine preparation. During the AC-FET, abnormal estradiol levels may modulate the degree of trophoblastic invasion and extent of vascular remodeling at the time of implantation, resulting in a latter exuberant trophoblastic growth (Kaser et al., 2015). In this review we found no differences in the risk of placenta previa between NC-FET and AC-FET groups; therefore, the increased incidence of placenta accreta in the AC-FET population does not appear to have been influenced by this factor.

Clinical considerations

Evaluating the safety of an intervention is critically important when balancing benefits against harm. Therefore, maternal and perinatal outcomes should be considered when making treatment decisions; however, these are rarely reported. In the FET population, we should consider maternal and perinatal risks when we decide on the endometrium preparation method, since NC-FET patients harbor decreased risks for obstetrical complications, as we found in this study.

The results of the present study encourage the use of NC-FET cycles, whereas AC-FET cycles ought to be used only when ovulation fails. The high rates of HDP show similarities with oocyte donation pregnancies, a known high-risk population for these and other maternal adverse outcomes in ART (Giannakou et al., 2018; Moreno-Sepulveda et al., 2019).

From the data obtained in our meta-analysis, practitioners can increase the safety of their interventions; identifying those patients who potentially require additional care. AC-FET patients will benefit with single embryo transfer, avoiding the risks of multiple pregnancies. Also, their obstetricians should implement adequate monitoring strategies during prenatal, labor and postnatal care.

Future research

A large, multisite RCT comparing pregnancy outcomes between NC-FET and AC-FET would be the optimal method to validate our findings.

Further research should, if confirming these results, clarify the role of corpus luteum and its compounds on perturbed maternal cardiovascular function and placentation in order to replace

these in patients undergoing FET cycles in which NC-FET is not possible to perform, such as non-ovulatory women.

In addition, we must be aware of epigenetic reprogramming in very early development and its relation to ART techniques. Follow-up studies on children born after ART should be performed throughout their life to monitor the development of adult diseases. Future meta-analyses should involve uncommon neonatal and childhood outcomes, aiming at providing more reliable data on the impact of cryopreservation.

Conclusions

Pregnancies after NC-FET have a more favorable outcome compared to AC-FET, with lower rates of HDP, preeclampsia, LGA and macrosomia. The development of gestational hypertension in FET cycles seems not to be influenced by the mode of endometrial preparation. This is very valuable information, since an increasing number of FET cycles are performed, including the "freeze-all" strategy. Future studies are required to clarify the underlying biologic mechanisms of our findings, and further RCTs are needed to improve the quality of evidence.

Authors' roles

J.M. and M.C. designed the study and collected the data. J.M. and J.E analyzed the data and drafted the first manuscript. All authors interpreted the pooled data, critically revised the manuscript for important intellectual content and approved the final version.

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Conflict of interest

The authors have no conflict of interest to declare.

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