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Frozen-thawed embryo transfer: the potential importance of the corpus luteum in preventing obstetrical complications

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Abstract

The use of frozen-thawed embryo transfer (FET) has increased over the past decade with improvements in technology and increasing live birth rates. FET facilitates elective single-embryo transfer, reduces ovarian hyperstimulation syndrome, optimizes endometrial receptivity, allows time for preimplantation genetics testing, and facilitates fertility preservation. FET cycles have been associated, however, with an increased risk of hypertensive disorders of pregnancy for reasons that are not clear. Recent evidence suggests that absence of the corpus luteum (CL) could be at least partly responsible for this increased risk. In a recent prospective cohort study, programmed FET cycles (no CL) were associated with higher rates of preeclampsia and preeclampsia with severe features compared with modified natural FET cycles. FET cycles are commonly performed in the context of a programmed cycle in which the endometrium is prepared with the use of exogenous E_2 and P. In these cycles, ovulation is suppressed and therefore the CL is absent. The CL produces not only E_2 and P, but also vasoactive products, such as relaxin and vascular endothelial growth factor, which are not replaced in a programmed FET cycle and which are hypothesized to be important for initial placentation. Emerging evidence has also revealed other adverse obstetrical and perinatal outcomes, including postpartum hemorrhage, macrosomia, and post-term birth specifically in programmed FET cycles compared with natural FET cycles. Despite the widespread use of FET, the optimal protocol with respect to live birth rate, maternal health, and perinatal outcomes has yet to be determined. Future practice regarding FET should be based on high-quality evidence, including rigorous controlled trials.

Keywords

Frozen embryo transfer; preeclampsia; natural cycle; programmed cycle; pregnancy outcomes

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BENEFITS OF FROZEN EMBRYO TRANSFER

Frozen-thawed embryo transfer (FET) has increased dramatically over the past decade as the indications for the procedure have expanded, in part owing to improvements associated with vitrification compared with older slow-freeze methods(1). In the United States, embryo cryopreservation with subsequent FET has increased from 7.9% of cycles in 2004 to 40.7% in 2013 (2), with similar increases globally (3–5). Moreover, the use of the freeze-only strategy, with cryopreservation of all potentially viable embryos, has steadily increased in recent years (6, 7). This strategy facilitates elective single-embryo transfer, reduces the risk of ovarian hyperstimulation syndrome, and allows time for results from preimplantation genetic testing (PGT) to return. In addition, women are dramatically increasing their use of fertility preservation, necessitating the need for interval FETs (8). Further potential benefits of FET include a decrease in the incidence of low birth weight, small for gestational age, preterm birth, placenta previa, placental abruption, and perinatal mortality compared with fresh embryo transfer (ET) (9, 10). Although less convincingly proven, some studies have suggested that FET is associated with a higher live birth rate compared with fresh ET, potentially because of better endometrial receptivity associated with FET (6, 11).

PROTOCOLS USED FOR FROZEN-THAWED EMBRYO TRANSFER

Compelling data indicate that cryopreserved embryos must be transferred to the uterus during a critical endometrial window for establishment of pregnancy (12). Commonly used protocols for FET in ovulatory women are the natural cycle, modified natural cycle, stimulated cycle, and programmed cycle. With a natural cycle, a dominant follicle matures, producing E_2 which leads to development and thickening of the uterine lining (endometrium). Ovulation occurs naturally, and the ovulation site becomes the corpus luteum (CL), a functional ovarian cyst producing P which allows the endometrium to become receptive to implantation of the embryo. A modified natural cycle is very similar to the natural cycle, except that ovulation is triggered by injection of hCG rather than by the spontaneous LH surge, and luteal phase support with the use of P may be prescribed (3). In a stimulated cycle, ovulation is induced with either clomiphene citrate, letrozole, or gonadotropins, resulting in one or more CLs.

In contrast, in a programmed cycle, exogenous E_2 and P lead to development of the endometrium. The ovary is suppressed, and thus there is no development of a dominant follicle, ovulation does not occur, and there is no CL. The timing of the transfer is based on the number of days elapsed after initiation of exogenous P. Intramuscular P in oil is preferred to be administered because recent data support superiority of this route of administration over vaginal P in the absence of the CL (13). In clinical practice, the programmed cycle is popular because it involves less monitoring and the ET can be scheduled on a convenient day for the patient and the practice. Despite the widespread use of FET, the optimal protocol with respect to live birth rate and pregnancy outcome has yet to be determined (14, 15).

RISK OF HYPERTENSIVE DISORDERS OF PREGNANCY WITH FET

Multiple studies have demonstrated an increased risk for hypertensive disorders of pregnancy associated with in vitro fertilization (IVF) particularly with FET (6, 10, 16–22). Some of the studies reporting an increased risk of preeclampsia with IVF grouped both fresh (multiple CLs) and frozen-thawed (frequently absent CL) transfers together within the category of IVF (23–26). Most studies that directly compared fresh ET versus FET reported higher risk with FET for hypertensive disorders of pregnancy (10, 18–22).

Perhaps one could hypothesize that there is something different about transferring “second-string” embryos in an FET. Maybe in the available datasets, the better embryos were transferred first in a fresh cycle and those that remain for FET were more likely to lead to abnormal placentation. This “second-string” hypothesis is unlikely to be correct, given that hypertensive disorders were still noted to be higher with FET even when considering freeze-only cycles in which all embryos (including the best ones) were frozen. A meta-analysis performed by Roque et al. (6) including 11 studies with 5,379 patients reported a significant overall increase in elective FET compared with fresh frozen transfer (risk ratio [RR] 1.12, 95% confidence interval [CI] 1.01–1.24). The RR of preeclampsia was 1.79 (1.03–3.09) for elective FET versus fresh ET.

One may also hypothesize that perhaps FET has a higher risk of preeclampsia because of some differences among women who conceive via FET versus those who conceive via fresh ET. However, an elegantly designed Nordic study suggests that this is not the case (20). In that study, the investigators examined the risk of hypertensive disorders of pregnancy for women who conceived twice with the use of assisted reproductive technology (ART), with each woman essentially serving as her own control. The FET was always associated with a trend for a higher risk for hypertensive disorders, regardless of whether the FET led to the first or the second pregnancy. If both pregnancies for an individual woman were conceived with FET, the risk for hypertensive disorders remained elevated in both the first and the second pregnancy.

Overall, the literature strongly suggests that FET increases the risk of preeclampsia. But the reasons for this increased risk are not clear. Over a decade ago, Kirk Conrad proposed the hypothesis that absence of the CL in the programmed cycles that are commonly used for FET may lead to an increased risk of abnormal maternal cardiovascular adaptation to pregnancy and subsequent increased risk of preeclampsia (27).

Maternal Cardiovascular Adaptation and the Corpus Luteum

Why might absence of the CL in programmed cycles increase the risk for hypertensive disorders of pregnancy? This hypothesis is biologically plausible because the CL produces not only E_2 and P, but also vasoactive products such as relaxin, vascular endothelial growth factor (VEGF), and angiogenic metabolites of estrogen (28–30). Before the establishment of the placenta as a source of pregnancy-maintaining reproductive hormones, such as P and estrogen, the CL serves as an important source of reproductive hormones. Specifically, the vasoactive products of the CL are hypothesized to be important for initial placentation, and abnormal early placentation has been proposed to be a critical step in the development of

preeclampsia (31–35). Because the relaxin and VEGF are not replaced, the programmed cycle is associated with a deficiency of these vasoactive products compared with natural, modified natural, and stimulated cycles.

Findings from studies in nonhuman animal models and early studies in humans supported the hypothesis that absence of the CL could be important for maternal cardiovascular adaptation to pregnancy. Circulating relaxin is one biologically plausible mediator for any effect of absent CL (36, 37). Relaxin is secreted solely from the CL during human pregnancy (28, 29). Relaxin is a potent vasodilator (38–40) that mediates circulatory changes, including increases in glomerular filtration rate (GFR) and effective renal plasma flow (eRPF), cardiac output, and arterial compliance in the gravid rat model (41, 42) and possibly in pregnant women too, as shown in one pilot study (43). In a gravid rat model, rat relaxin-neutralizing antibody (MCA1) and control antibody (MCAF) was used in virgin and day 11 pregnant rats. The neutralizing antibody completely inhibited the gestational increase in GFR and eRPF, as well as the reduction in effective renal vascular resistance, on gestational days 11 and 14 (MCA1 vs. MCAF: $P< 0.01$; $P< 0.05$ vs. MCA1 and MCAF virgin.). This was coupled with abolishment of the reduction in myogenic reactivity in small renal arteries from pregnant rats ex vivo (27). Other vasoactive products of the CL, such as VEGF, are also not replaced during a programmed FET, and their absence could have consequences. In fact, we know little about which factors are secreted into the circulation by the CL in women that may be important for pregnancy health and are not replaced in FET protocols with absent CL.

Increasing arterial compliance is a major physiologic adaptation in normal human pregnancy. Therefore, carotid-femoral pulse-wave velocity and transit time were assessed by investigators at the University of Florida (44).The investigators reported an attenuation of the expected decline in carotid-femoral pulse-wave velocity and rise in the carotid femoral pulse-wave transit time during the first trimester between 0-CL and combined single/multiple-CL cohorts (group-time interaction: $P=.06$ and $P=.03$, respectively). The same changes from before pregnancy in the 0-CL cohort were most striking at 10–12 weeks of gestation ($P=.01$ and $P=.006$ vs. 1 and >1 CL, respectively).

In a parallel study (45) conducted with a different population of women at Stanford University (n = 85), women with absent CL (programmed cycle) did not have the expected drop in mean arterial blood pressure compared with those with 1 CL. In FET cycles, a lower reactive hyperemia index and a higher augmentation index was noted in FETs without a CL compared with FETs in a natural cycle with a CL (both $P=.03$). In FETs, the numbers of angiogenic and nonangiogenic circulating endothelial progenitor cell numbers were lower in the absence of a CL ($P=.01$ and $P=.03$).

In another study of 184 infertile women at Stanford University (30), levels of relaxin-2, creatinine, and electrolytes were assessed in early pregnancy. Relaxin-2 levels were undetectable in patients who had undergone programmed FET with absence of the CL. Creatinine, sodium, and total CO_2 levels were significantly higher in the 0-CL group (relaxin absent) compared with all other groups (relaxin present), which was consistent with the findings by Smith et al. (43). These findings suggest potential compromise of the normal

renal and osmoregulatory changes of pregnancy in the absence of a CL that could contribute to the higher risk of adverse pregnancy outcomes such as preeclampsia.

Overall, these studies support the premise that absence of the CL is associated with deficient circulatory adaptations during early gestation. Such findings are of concern because multiple studies demonstrated that abnormal maternal vascular adaptation to pregnancy is linked to adverse pregnancy outcomes, including preeclampsia (31–35).

Observational Data Regarding Risk of Hypertensive Disorders with FET in the Presence or Absence of the CL

As noted above, multiple studies have demonstrated an increased risk for hypertensive disorders of pregnancy associated with IVF (15, 23, 24, 46), and particularly with FET (6, 10, 16–22) Importantly, most studies that directly compared fresh ET and FET reported higher risk of hypertensive disorders of pregnancy with FET (10, 17, 19–21). However, many of these early reports did not specify the protocol used to prepare the endometrium.

More recent studies have included details regarding FET protocol. An observational prospective cohort study was the first to report the incidence of preeclampsia with the use of a modified natural cycle compared with the programmed cycle. Obstetrical outcomes for singleton live births with autologous oocytes were compared between groups by number of CLs (44). Women were enrolled at 8 weeks' gestation, and pregnancy outcomes were adjudicated by review of medical records by an obstetrician blinded to fertility treatment group. Programmed FET cycles with absence of the CL were associated with higher rates of preeclampsia (12.8% vs. 3.9%; P=.02) and preeclampsia with severe features (9.6% vs. 0.8%; P=.002) compared with modified natural FET cycles (1 CL). Regression analysis controlled for nulliparity, age, history of hypertension, body mass index, diabetes mellitus (pregestational and gestational), and polycystic ovary syndrome. Absence of the CL was predictive of preeclampsia (adjusted odds ratio [OR], 2.73, 95% CI 1.14–6.49) and preeclampsia with severe features (OR 6.45, 95% CI 1.94–25.09) compared with a single CL (with the 1-CL group including spontaneous conceptions among subfertile women as well as modified natural-cycle FET). In the analysis restricted to FET cycles, the programmed FET cycle was associated with higher risks for preeclampsia (adjusted OR 3.55, 95% CI 1.20–11.94; P=.03) and preeclampsia with severe features (adjusted OR 15.05, 95% CI 2.59–286.27; P=.01) compared with a modified natural-cycle FET.

A large observational study in Sweden (9) examining the risk of hypertensive disorders in fresh ET versus FET confirmed these findings regarding a higher risk for hypertensive disorders in the absence of the CL. That study included singletons and twins and compared autologous and donor oocyte, linking data from the ART registry with hospital discharge records and birth certificates. Among pregnancies conceived with autologous eggs resulting in singletons, the incidence of preeclampsia was greater after FET versus fresh ET (7.51% vs. 4.29%, adjusted OR 2.17, 95% CI 1.67–2.82). Preeclampsia without and with severe features, preeclampsia with preterm delivery, and chronic hypertension with superimposed preeclampsia were more frequent after FET versus fresh ET (3.99% vs. 2.55%, 2.95% vs. 1.41%, 2.76 vs. 1.48%, and 0.95% vs. 0.43%, respectively). Among pregnancies from autologous eggs resulting in twins, the frequencies of preeclampsia with severe features

(9.26% vs. 5.70%) and preeclampsia with preterm delivery (14.81% vs. 11.74%) were higher after FET versus fresh ET. Among donor egg pregnancies, rates of preeclampsia did not differ significantly between FET and fresh ET: 10.78% vs. 12.13% for singletons and 28.0% vs. 25.15% for twins (19). In that study, the specific protocols used for the autologous FET cycles were not specified. In general, nearly all donor-oocyte recipient cycles use a programmed cycle.

Saito et al. in Japan (47) also examined the risk for hypertensive disorders in autologous pregnancies in a large epidemiologic study with details regarding the FET protocols used (natural cycle: n = 29,760; programmed cycle: n = 75,474). They determined that compared with a natural-cycle FET, pregnancies after programmed FET had increased odds of hypertensive disorders of pregnancy (adjusted OR 1.43, 95% CI 1.14–1.80) and placenta accreta (adjusted OR 6.91, 95% CI 2.87–16.66) and decreased odds for gestational diabetes mellitus (adjusted OR 0.52, 95% CI 0.40–0.68) compared with pregnancies after natural-cycle FET. The pregnancy and live birth rates were significantly lower in the programmed versus natural-conception cycles.

Another recent observational study in the United States suggested a higher risk of hypertensive disorders in donoroocyte fresh and FET cycles as well as in autologous FET cycles. Luke et al. (46) reported that risk for hypertensive disorders of pregnancy was increased for autologous thawed (1.30 [1.20–1.40]), donor fresh (1.92 [1.71–2.15]), and donor thawed (1.70 [1.47–1.96]) cycles. No increase was seen in fresh autologous IVF cycles, treatment that occurs in the presence of multiple CLs.

OTHER OBSTETRICAL COMPLICATIONS ASSOCIATED WITH FROZEN EMBRYO TRANSFER

A meta-analysis performed by Sha et al. (10) included 31 studies, and a funnel plot (a scatterplot of treatment effect against a measure of study precision) found that the pregnancies resulting from FET were associated with lower relative risks of placenta previa (RR 0.61, 95% CI 0.43–0.88), placental abruption (RR 0.63, 95% CI 0.47–0.85), and low birth weight (RR 0.74, 95% CI 0.69–0.79) compared with fresh ET. However, pregnancies resulting from FET were associated with increased risks of pregnancy-induced hypertension (RR 1.44, 95% CI 1.16–1.78), postpartum hemorrhage (RR 1.28, 95% CI 1.14–1.44), and large for gestational age (RR 1.58, 95% CI 1.31–1.90) compared with fresh ET. In general, children born after FET have been found to be larger for gestational age and macrosomic (>4,500 g) compared with both fresh ET cycles and spontaneous conception (22, 48–50). The explanation for these findings remains unknown, with little data examining FET protocol choice and these other obstetrical outcomes.

The latest observational data assessing neonatal and maternal outcomes after FET have suggested higher rates of hypertensive disorders of pregnancy, postpartum hemor-rhage, post-term birth, and macrosomia specifically in programmed FET cycles compared with natural and stimulated cycles (9). These findings suggest a plausible association between absence of CL in FET cycles and adverse obstetrical outcomes. Given the increasing use of

FET, there is a critical need to determine if elements of the treatment, including specific FET protocols, could be modified to optimize outcomes.

FUTURE DIRECTIONS

To further determine the impact of natural versus programmed FET protocol on the rate of preeclampsia, well designed randomized controlled trials are warranted. A multicenter parallel-group superiority randomized control trial of FETs in women aged 18–39 years to determine incidence of preeclampsia and live birth rate in modified natural versus programmed cycles will soon be initiated. The eligibility criteria for the clinical trial will need to mirror the general population seeking FET. Women with regular menstrual cycles undergoing autologous elective single-FET will be randomized to either a modified natural cycle (CL present) or a programmed cycle (CL absent) with the use of a stratified randomization design to balance the use of PGT across the two treatment arms. This study will compare the rates of preeclampsia and live birth between natural and programmed FET cycles.

CONCLUSION

In recent years, use of FET cycles has increased, including freeze-only strategies of potentially viable embryos. FET cycles facilitate single-FETs, reduce the risk of ovarian hyperstimulation syndrome, and allow time for PGT results to return. However, FET cycles have been associated with an increased risk for hypertensive disorders of pregnancy, with recent findings suggesting that the absence of the CL in programmed cycles may play a role in this increased risk. The magnitude of emerging evidence regarding increased risk of obstetrical outcomes when comparing programmed FET cycles and natural cycles is sufficient to warrant clinical concern and potential changes in practice. Future studies examining the maternal and perinatal outcomes associated with FET should specify which protocols were used. A rigorous randomized controlled trial of natural versus programmed FET protocols is warranted, because there are advantages to programmed FET, such as flexibility in scheduling. The findings from future studies will allow physicians to optimize the outcomes for millions of women undergoing FET cycles globally.

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