Fresh embryo transfer results in altered placental epigenetic regulation: a rationale for frozen embryo transfer

Because assisted reproductive technologies (ARTs) have become increasingly effective, much attention has shifted toward the safety and outcomes of children born from these techniques. For comparing the outcomes of different methodologies at each step in the ART process, 1 step noted to have a differential effect is the timing of embryo transfer (ET), whether it is a fresh ET or a frozen embryo transfer (FET). Indeed, several publications have reported differing rates of low birth weight, prematurity, abnormal placentation, and gestational hypertension or pre-eclampsia between these 2 ET modalities (1, 2).

It has been hypothesized that the potential for embryo-endometrial asynchrony in fresh ET, which can be controlled for during a delayed FET, may be responsible for some of these differences. This may be a result of the supraphysiologic levels of circulating hormones stemming from ovarian stimulation and a premature rise in progesterone level observed in fresh ET cycles. Previous studies have evaluated differences in methylation patterns and epigenetic regulation between ART-conceived pregnancies and naturally conceived pregnancies. However, none have analyzed the difference between fresh ET and FET, which could provide additional insight into the mechanism by which this altered endocrinology affects the aforementioned outcomes.

That is why a study published in this issue of the journal is of such interest. Barberet et al. (3) compared DNA methylation patterns of imprinted genes (IGs) and transposable elements (TEs) in placental tissue and cord blood of pregnancies resulting from fresh ET, FET, and natural conception, wherein natural conception served as a control group. The results showed that the DNA methylation patterns were altered in the fresh ET group compared with those in the naturally conceived controls, whereas there was no difference between the FET and control groups.

In a 2012 systematic review and a meta-analysis of 11 studies, the rates of antepartum hemorrhage, low birth weight, small-for-gestational-age newborns, prematurity, and perinatal mortality were observed to be lower after FET than after fresh ET (1). Another systematic review and meta-analysis of 6 articles published in 2018 aimed at comparing obstetric outcomes showed that FET was associated with higher rates of placenta accreta, pregnancy-induced hypertension, and pre-eclampsia (2). These and other publications highlight the differences in placentation and birth weight, the latter potentially stemming from the former.

In an attempt to elucidate the mechanism by which ART affects placentation and fetal growth, it has been hypothesized that differential epigenetic reprogramming may play an important role. The 3 IGs evaluated in this study with a potential effect on placentation and birth weight have been previously shown to alter DNA methylation or expression patterns in humans as a result of ART: *H19*, *KCNQ1OT1*, and *SNURF*. For instance, the deletion of *H19* in mice—an IG widely present in placental tissue—led to placentomegaly and fetal growth control impairment (4). Although these differences in DNA methylation and expression patterns have been observed between ART-conceived and naturally conceived pregnancies and may, indeed, play an important role, the specific effect of fresh ET and FET on these patterns is yet to be clarified.

The publication in this issue by Barberet et al. (3) is the first to compare the methylation patterns of these IGs and TEs between fresh ET and FET and is, therefore, a valuable contribution to the existing literature on this topic. The investigators reported lower methylation levels of *H19* or insulin-like growth factor 2 in fresh ET than in FET and lower DNA methylation rate for long interspersed nuclear element 1, a TE that might be relevant for placental function. The investigators also proposed mechanisms that could account for the observed differences in the reproductive outcomes between these 2 types of transfers. There were no differences between FET-conceived and naturally conceived pregnancies, suggesting that ovarian stimulation results in an abnormal embryo-endometrial relationship. Moreover, given that the observed changes took place only in the placental samples and not in the cord blood samples, they hypothesized that although fresh ET may have an effect on placentation and related outcomes, it does not appear to affect the methylation pattern of these same genes in embryonic cells.

There are several limitations to the data presented. The study included only fresh ET and FET performed on day 2 or 3, which might limit its generalizability to the large number of blastocyst transfers being performed in current practice. One concern is that the data that shows further in vitro embryonic development may in itself have a significant effect on epigenetic regulation, particularly in cases of sequential culture in which the culture media is changed after day 3 of embryo development. Furthermore, as noted by the investigators in their discussion, the potential effect of embryo micromanipulation, such as during intracytoplasmic sperm injection, assisted hatching, or embryo biopsy, is difficult to assess in this study because embryos conceived via conventional in vitro fertilization and intracytoplasmic sperm injection were grouped together.

Although the limitations mentioned above must be considered, the investigators provided a very valuable contribution to the literature, identifying important epigenetic changes in the placental tissue of pregnancies conceived via fresh ET compared with those conceived via FET and naturally conceived pregnancies. This ongoing line of research promises to shed some light on the differences between fresh ET and FET and, perhaps more importantly, on the mechanisms by which these processes affect

the placentation and fetal growth of the resulting pregnancies. It is important to note that the lack of significant changes when comparing FET-conceived newborns with naturally conceived newborns strengthens the evidence suggesting the safety of FET and reinforces the trend toward freeze-all protocols.

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REFERENCES

1. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril 2012;98:368–77.
2. Roque M, Valle M, Sampaio M, Geber S. Obstetric outcomes after fresh versus frozen-thawed embryo transfers: a systematic review and meta-analysis. JBRA Assist Reprod 2018;22:253–60.
3. Barberet J, Romain G, Binquet C, Guilleman M, Bruno C, Ginod P, et al. Do frozen embryo transfers modify the epigenetic control of imprinted genes and transposable elements in newborns as compared with fresh embryo transfers and natural conceptions? Fertil Steril 2021;116:1468–80.
4. Eggenschwiler J, Ludwig T, Fisher P, Leighton PA, Tilghman SM, Efstratiadis A. Mouse mutant embryos overexpressing IGF-II exhibit phenotypic features of the Beckwith-Wiedemann and Simpson-Golabi-Behmel syndromes. Genes Dev 1997;11:3128–42.