Frozen embryo transfer and preeclampsia: where is the link?

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Purpose of review
Preeclampsia is the leading cause of maternal death and has a higher incidence in patients undergoing assisted reproduction treatments. New studies about the mechanisms by which it is more frequent in this population have emerged. The purpose of this review is to gather current information about the available results on this correlation and its possible physiopathology.

Recent findings
Recent publications on the physiopathology of preeclampsia indicate that the corpus luteum is the main source of hormonal production until placental formation and that apart from the secretion of estrogen and progesterone, corpus luteum also produces important substances involved in maternal circulatory adaptation, such as relaxin.
With the recent increasing number of frozen embryos transfer in natural cycles or under hormonal replacement, this adaptive circulatory process may be unbalanced and predispose this population to preeclampsia.

Summary
This article provides a review of frozen embryo transfer and available protocols, the highest incidence of gestational hypertensive disorders presented by the infertile population and an overview of the possible impact of the absence of corpus luteum on the genesis of this disease.

Keywords
corpus luteum and relaxin, frozen embryo transfer, gestational circulatory adaptation, preeclampsia

INTRODUCTION

The use of assisted reproduction treatments (ART) has progressively expanded in contemporary medicine. With the advent of vitrification, better survival rates after thawing have been reported, and thus, a greater number of embryos undergo this process. Frozen embryo transfer (FET) can be performed in a natural cycle or in under hormonal replacement protocols. Recent studies associate perinatal outcomes in pregnant patients after ART with a higher incidence of gestational hypertensive disorders, such as preeclampsia, and severe preeclampsia. These results are even more evident in patients undergoing frozen embryo transfer, especially when preceded by cycles of artificial endometrial preparation. In these cycles, estrogen and progesterone are usually replaced, without the development of corpus luteum. Considering that corpus luteum secretes other substances that contribute to physiological circulatory adaptation in the first trimester of pregnancy, its absence could be a key player.

COMPARISON BETWEEN FRESH VERSUS FROZEN EMBRYO TRANSFER

Pregnancies resulting from ART are known to be associated with gestational and neonatal diseases for years, mainly because of its relationship with multiple pregnancy and its associated risks of obstetric complications, but also to other factors associated such as maternal age and/or primiparity. Despite the improvements in laboratory techniques, vitrification and the current trend of transferring only one embryo to minimize the twin gestation rates, still adverse obstetric events in this group persist. These events include placental changes and gestational hypertensive disorders [1,2,3,4].

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KEY POINTS

- Frozen embryo transfer can be performed in natural cycles or under hormonal replacement therapy.
- Recent studies have shown a higher incidence of gestational hypertensive disorders – specifically preeclampsia – after frozen embryo transfer, but this is not observed when the transfer is done in a natural cycle.
- The corpus luteum secretes important substances that contribute to physiological circulatory adaptation in the first trimester of pregnancy.
- The absence of corpus luteum may represent an important contribution to the increase of preeclampsia and severe preeclampsia in the infertile population.

The consequences of using cryopreserved oocytes/embryos in ART and their possible maternal/fetal consequences are critical motivations for current reviews. Studies suggest a connection of higher rates of obstetric disease development with FET when compared with fresh embryo transfers, especially related to large for gestational age babies (LGA), postpartum hemorrhage, and hypertensive pregnancy disorders [5,6,9].

On the other hand, initial studies showed better pregnancy and live-birth rates with FET, which prompted a trend toward freezing all embryos prior to transfer in a strategy called 'freeze-all' [6,7,8,9]. However, these initial studies were soon confronted with different evidence [10].

In 2018, Zhang *et al.* [11] compared 4112 single full-term births between FET and fresh embryo transfer, finding a lower percentage of small for gestational age and low birth weight when using frozen embryos. The authors suggested that vitrification could have an impact on embryo metabolism, which might explain these results [11].

Other studies have also shown higher chances of LGA newborn after FET. The real reasons behind this increase are questionable, encouraging us to reflect on whether freezing may or may not influence the placentation and embryo epigenetic development process resulting in a distinct to physiological gestational environment [5].

A retrospective cohort study published in 2017 attempted to prove the relationship between the use of frozen embryos with an increased risk of developing preeclampsia analyzing 15 937 births. The results of the single embryo transfer showed a prevalence of preeclampsia of 7.5% in patients undergoing FET, compared with 4.3% in the fresh embryo transfer group. These findings may be related to the negative impact of high synthetic estrogen dosages used in endometrial preparation of FET cycles. These high hormonal concentrations can interfere with embryonic adhesion and trophoblast invasion, contributing to the pathogenesis [12]. Similar results had already been presented by Chen *et al.* [13] in 2016, when patients with polycystic ovary syndrome were analyzed showing an incidence of preeclampsia 4.4% in FET compared with 1.4% in the group with fresh embryo transfer [13].

Better pregnancy and live-birth rates were also reported in 2019 using vitrified blastocysts [9]. These findings seem to correspond to the negative effects of high dosages of exogenous gonadotropins used and elevated steroid levels obtained after the ovarian stimulation circle. However, complications such as increased risk of hypertensive disorders and placental complications also seem to be more evident in this population. Researchers warn of the possible impact of vitrification on embryonic epigenetics and its contribution to hypertensive disorders [7,9].

To better understand the impact of freezing/thawing on birth-weight, we performed a retrospective analysis in 360 women who had two newborns singleton after oocyte donation cycle, first baby after a fresh embryo transfer and the second baby with the frozen embryo from the same donation. Their birth weight was comparable (3.184 versus 3.226 g) between fresh or frozen embryo replacements. Thus, other concepts may contribute such as ovarian stimulation or the protocol to prepare the patient for embryo replacement [14].

WHICH IS THE BEST PROTOCOL FOR FROZEN EMBRYO TRANSFER?

There is no consensus on the best protocol to perform FET yet, but three of them are considered the main options: frozen embryo transfer in natural cycle, modified natural cycle (MNC), and hormonal replacement cycles (HRT) [15].

MNC differ from natural cycle by the use of Human chorionic gonadotropin (HCG) to trigger final oocyte maturation instead of relying on endogenous Luteinizing hormone (LH) peak measured in blood or urine. In both cases, corpus luteum is present. However, these options present great difficulty in anovulatory patients with irregular menstruation cycles or in postmenopausal period. Artificial cycles are more predictable, being usually prepared with oral/transdermal estradiol and oral/vaginal/injectable progesterone. The variability of these protocols raises the question for further research and studies to identify the best option in live-birth rates, and the lowest rates of maternal/fetal adverse events [15,16▪].

DIFFERENCES IN RESULTS BETWEEN FROZEN EMBRYO TRANSFER PROTOCOLS

Natural cycle would prepare the endometrium relying on the endogenous, spontaneous LH peak. Modified natural cycle would avoid the uncertainty of the spontaneous LH surge by administering a dose of HCG. To elucidate if this HCG dose had any deleterious impact on pregnancy rates, Fatemi *et al.* [17] performed a small Randomized controlled trial (RCT). They compared 124 women undergoing FET in natural cycle with endogenous LH peak (61 women) with women using exogenous HCG (63 women). There was a considerable difference in the rates of ongoing pregnancy rates between the groups (31.1% in natural cycle versus 14.3% in MNC, $P = 0.025$), suggesting that the former has advantages when compared with MNC; thus, its use in women with regular menstrual cycles might be preferred. The group in natural cycle also had a higher frequency of medical visits for cycle monitoring, being a relevant negative point. However, sample size was modest and the study was underpowered [17].

The high concentrations of estrogen commonly used to prepare the endometrium in HRT may have a deleterious effect on endometrial functionality, which is not always translated by endometrial thickness. A retrospective study published in 2011 analyzed 611 healthy patients and found substantial differences in clinical pregnancy rates comparing FET in natural cycle versus HRT (41.9 and 30.4%, $P = 0.006$). Implantation and ongoing pregnancy rates were also substantially higher in the natural cycles (34.9 versus 24.3%, $P = 0.003$) and (38.1 versus 27.5%, $P = 0.013$). These results suggest that natural cycles seem to be more physiological and have advantages when compared with the preparation of exogenous hormones [18].

In 2016, 1482 cases of FET were analyzed retrospectively comparing pregnancy rates and perinatal outcomes between MNC and HRT in healthy women with regular menstruation. MNC had higher rates of ongoing pregnancy, implantation, and live births when compared with HRT. Interestingly, gestational complications such as preeclampsia were analyzed and did not differ between groups [19].

In a prospective cohort study published in 2017, Cerrillo *et al.* [20] analyzed the impact of exogenous endometrial preparation by comparing implantation rates, ongoing pregnancy rates, and live births among patients undergoing FET (530) in natural cycle (spontaneous LH peak), MNC (oocyte maturation with HCG), and HRT cycles. No statistically significant differences were found among the groups, but there was a higher prevalence of miscarriage in the artificial cycles when compared with HCG and endogenous LH cycles (21.2 versus 12.9 versus 11.1%, $P < 0.01$). The authors suggested that the HRT cycle is more comfortable for the patient and has better control for the attending gynecologist to program the FET, with lower number of visits to the clinic [20].

A more recent metaanalysis performed by Yarali *et al.* [15] established no substantial differences among the different protocols for FET regarding clinical pregnancy and live-birth rates. Apart from that, no scientific evidence between the compared treatments was found regarding obstetric and neonatal outcomes [15]. Similarly, a recent randomized clinical trial transferring embryos on the third day of development did not show relevant differences in pregnancy rates between the natural cycle or HRT protocols [21].

So, although in small studies it may seem preferable to transfer embryos in pure natural cycle, when take datasets are evaluated, the evidence is not so convincing, as all three protocols seen to be similarly efficient.

FROZEN EMBRYO TRANSFER IN ARTIFICIAL CYCLES AND HIGHER INCIDENCE OF PREECLAMPSIA

Preeclampsia is the main cause of maternal death affecting up to 4% of pregnancies and is commonly described as blood pressure at least 140/90 mmHg (in two measurements) after 20 weeks of gestation, with proteinuria more than 300 mg/24 h or injury to target organ/eclampsia eminence [22].

Recent publications suggest increased risk of gestational hypertensive disorders when performing FET in HRT for endometrial preparation [23▪▪]. Figure 1 demonstrates this high incidence in different studies. One of the hypotheses currently being discussed nowadays is that the absence of corpus luteum in these situations may be the cause, as the corpus luteum secretes many substances beyond estradiol and progesterone (i.e., steroids, cytokines, chemokines, growth factors). These substances include VEGF and relaxin [24]. The latter is secreted exclusively by corpus luteum and plays an important role in the circulatory adaptive system at endometrial and systemic levels. Its absence may be related to the difficulties imposed on the trophoblast invasion into the uterine spiral arteries, thus intervening in the placentation process and predisposing the patient to preeclampsia. Therefore, the replacement of the other factors secreted by the corpus luteum to mimic a natural cycle may be necessary, thus providing results and safety similar to spontaneous pregnancies [25▪].

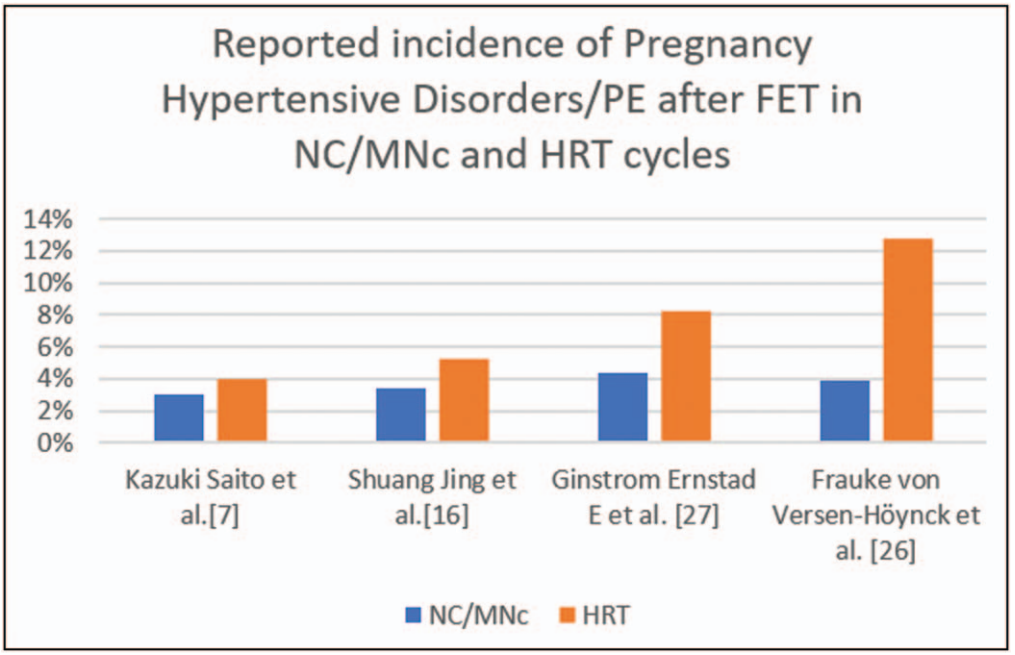

FIGURE 1. Frozen embryo transfer, natural cycle, modified natural cycle (MNC), hormonal replacement cycles (HRT). Regardless of the inclusion/exclusion criteria of the patients in each group, the concepts for gestational hypertensive disorders, or the number of embryos transferred, the incidence of gestational hypertensive disorders remained elevated in HRT compared with natural cycle/MNC.

Relaxin is a peptide hormone of approximately 6 kDa, member of the insulin/relaxin family [26]. It is detectable early in postovulatory periods and has high concentrations at the end of the first trimester of pregnancy. It is implied that this substance is important for the adaptive gestational hemodynamic by direct action and as a mediator of other regulatory substances. In animal models, its exogenous administration causes systemic vasodilation, increased intravascular volume and considerable increase in arterial compliance. These findings are similar to the physiologically adaptation in the first gestational trimester [26].

The use of relaxin secretion blocking substances, in mice models, interferes with the previously mentioned circulatory adaptive processes [27]. However, in the late stages of pregnancy, these adaptations are attenuated, as the absence of relaxin is compensated by the functional placenta. Its circulatory effects are mainly related to the secretion of nitric oxide, a potent vasodilator that stimulates its production in endothelial vascular walls. However, the effect on arterial compliance is because of the action on the tone of vascular smooth muscle and its structural components (endothelial extracellular matrix). Despite the primary local of relaxin action being the endometrium, the relaxin and its receptor relaxin/insulin-like family peptide receptor 1 are expressed in a variety of female reproductive organs [27].

Studies on their angiogenic role at the endometrium suggest that their deficiency may be related to the unbalanced process of fusion of spiral arterioles in trophoblast invasion, predisposing the patient to preeclampsia [27]. Uterine antiapoptotic effects are also reported, and accelerated placental apoptosis within decreased serum relaxin concentrations could contribute to the genesis of gestational hypertensive disorders. Apart from the local arterial activity regulation of vascular endothelial growth factor (VEGF), its administration could counteract the protein sFlt1 (Soluble fms-like tyrosine kinase-1) deleterious antiangiogenic effect, thereby balancing the endometrial angiogenesis [27].

A retrospective cohort study published in 2019 analyzed 11.037 FET cycles and aimed to compare adverse outcomes between natural cycle and AC. The results showed an increased incidence of hypertensive disorders of pregnancy in the FET group in HRT when compared with natural cycle (5.2 versus 3.4%, $P = 0.016$). These increased results also persisted when multiple pregnancies were analyzed (13.9 versus 6.4%, $P < 0.001$). These findings may be because of the negative impact of high hormonal concentrations during endometrial preparation on AC, whether in incorrect embryonic implantation, in the unbalanced secretion of important endometrial substances during pregnancy or in the interference with placental mechanisms [16▪].

Considering the corpus luteum function in the substances secretion that plays an important role in maternal circulatory adaptive response, Frauke Von Versen-Höynck led a pioneering study in 2019. Applanation tonometry was used to evaluate waveforms (PWV) and the carotid-femoral and carotid-radial transit-time to estimate arterial compliance and vascular resistance in pregnant women. The results showed a lower circulatory adaptive capacity in pregnant women who received embryos in artificial endometrial preparation cycles. These values were translated into a higher incidence of preeclampsia (12.8 versus 3.9%, $P = 0.02$) and severe preeclampsia (9.6 versus 0.8%, $P < 0.001$) in programmed cycles compared with modified natural cycle. In this study, the presence of multiple corpus luteum was not a statistically relevant risk factor for hypertensive disorder of pregnancy, suggesting that hormonal supraphysiological levels are not predisposing factors for hypertensive diseases [28▪▪].

Supraphysiological progesterone levels also appear to be associated with excessive trophoblast invasion and infraphysiological concentrations with insufficient decidualization process. A recent retrospective Japanese cohort study evaluated the transfer of frozen autologous embryos at 574 assisted reproduction clinics in this country. A comparison was made between FET in MNC/natural cycle versus AC. The pregnancy rate was significantly lower in the AC group (32.1 versus 36.1%, $P = <0.001$). Hypertensive disorders were present in 3% of patients undergoing FET in natural cycle and 4% of patients in AC, even though the latter group had a higher average age (38.0 versus 37.2, $P = <0.001$), recognized risk factor for these diseases. This study highlights the relationship between the type of protocol used for endometrial preparation and the incidence of hypertensive disease [7].

Similar results were reported in another recent study published in 2019 conducted in Sweden using 9726 FET and 24 365 fresh embryo transfers. The researchers analyzed the impact of the protocol used to perform the FET on the incidence of preeclampsia, comparing them with each other and with fresh cycles. The results also demonstrated a higher prevalence of preeclampsia and hypertensive disorders of pregnancy in the embryo transfer group at programmed cycles. These cycles do not have corpus luteum and this fact seems to play an important role in the disease's development. The percentage of preeclampsia in FET in programmed cycles was 8.2%, compared with 4.3% in stimulated cycles and 4.4% in natural cycles. Both presented higher incidence when compared with the prevalence of the disease in fresh embryos (3.7%) [23▪▪].

CONCLUSION

Vitrification is a major improvement used in infertility treatments. It has a variety of indications and although its use increases progressively, its impact on embryo metabolism and epigenetics remains uncertain.

Women undergoing infertility treatments have an increased incidence of gestational hypertensive disorders. Among this group, FET presents higher percentages of this disease when compared with fresh embryo transfer. These risks are accentuated when FET is performed in artificial endometrial preparation cycles (with no corpora lutea). Current studies suggest an important role of the absence of corpus luteum in genesis of these hypertensive disorders, may be related with the lack of products physiologically secreted by the corpus luteum.

Among these substances, progesterone and relaxin deserve special mention. Both affect the maternal circulatory adaptive process, on the trophoblast invasion process and on the decision-making process. Decreased concentrations of these substances can negatively affect maternal blood pressure, especially in the first trimester.

We believe that well designed prospective studies shed some light about these early findings, which if confirmed will stimulate us to prioritize wherever possible the transfer of embryos in natural cycles, especially in high-risk groups of patients.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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