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**Letter to the Editor**

# Artificial cycle frozen embryo transfer and obstetric adverse outcomes: association or causation?

Dear Sir,

We read with great interest the article entitled 'Obstetric and neonatal outcomes after natural versus artificial cycle frozen embryo transfer and the role of luteal phase support: a systematic review and meta-analysis' published in *Human Reproduction Update* and we sincerely congratulate the authors for their comprehensive data synthesis (Zaat *et al.*, 2023).

In this study, the principal analysis showed an increase in normal range birthweight and a decrease in large for gestational age, macrosomia, low birthweight, early pregnancy loss, preterm birth (PTB), very PTB, hypertensive disorders of pregnancy (HDP), preeclampsia (PE), placenta previa and postpartum hemorrhage (PPH) in natural cycle frozen embryo transfer (NC-FET) compared to artificial cycle FET (AC-FET). In an exploratory analysis, authors also observed that the use of luteal phase support (LPS) in NC-FET is associated with a decrease in PTB risk when NC-FET with or without LPS is compared to AC-FET (Zaat *et al.*, 2023). Limited data reported in this and previous studies suggest no differences in obstetric and neonatal complications between total NC-FET and modified NC-FET (Asserhøj *et al.*, 2021; Busnelli *et al.*, 2022; Zaat *et al.*, 2023).

Data from national and supranational registries demonstrate that the number of frozen cycles is progressively increasing and currently accounts for almost 40% of all IVF cycles performed (European IVF Monitoring Consortium (EIM), for the European Society of Human Reproduction and Embryology (ESHRE) *et al.*, 2022). Although AC regimens were initially developed for women who were anovulatory, they are now adopted also in women who ovulate regularly (von Versen-Höynck and Griesinger, 2022). However, the high number of adverse obstetric outcomes that have been demonstrated to be associated with AC-FET makes one perceive this endometrial preparation strategy as potentially responsible for a severe obstetric syndrome. Accordingly, both von Versen-Höynck and Griesinger (2022), in a recent opinion paper published in *Human Reproduction*, and Zaat *et al.* (2023), in their meta-analysis, discourage the use of AC regimens for FET in ovulatory women or women capable of ovulation, as these may cause a strong deviation from physiology and put patient and fetus at avoidable health risk, without any apparent health benefit (von Versen-Höynck and Griesinger, 2022; Zaat *et al.*, 2023). Unfortunately, the progressive reduction of AC-FET would also have negative consequences. In fact, abandoning the AC regimens would mean renouncing their main advantage which is also the reason behind their widespread use: i.e. the opportunity to program the thawing and transferring of embryos according to the needs of the IVF laboratory, the treating doctors and the patient (Groenewoud *et al.*, 2018; von Versen-Höynck and Griesinger, 2022). This is particularly relevant in IVF clinics that perform a high number of cycles every year. In these settings, cycle programming allows avoidance of a high volume of work on certain days of the week to ensure that all patients receive the same quality of treatment and attention, and that the IVF staff can focus on each individual patient in the same manner. Moreover, incubator overusage and door openings could be minimized, thus reducing the negative effect on embryo development caused by carbon dioxide and temperature variations (Garcia-Velasco and Fatemi, 2015). Suggesting the total abandonment of a therapeutic regimen on the basis of associations that emerged from pooling of retrospective data is in disagreement with the rules of epidemiological research. To account for possible confounders, in a previous systematic review and meta-analysis, we conducted sub-analyses by pooling only risk estimates adjusted for both preconception and IVF covariates. Applying this method, we obtained results partially different from those of Zaat *et al.* (2023). Indeed, we showed that the 'only' adverse obstetric outcomes that were significantly more frequent in AC-FET pregnancies compared to NC-FET pregnancies even after adjusting the analyses for possible confounders were: HDP, PE, PPH and cesarean section (Busnelli *et al.*, 2022). Herein, we have tried to go one step further: we have re-analyzed the results of Zaat *et al.* to clarify the causal relationship between the exposure factor (i.e. AC-FET) and the obstetric and perinatal outcomes. Specifically, in order to infer causality, we adopted the Bradford Hill's criteria and the methodology suggested by the Continuous Update Project (CUP) of the World Cancer Research Fund (Hill 1965; Schünemann *et al.*, 2011; Tsilidis *et al.*, 2023). This approach allowed us to precisely delineate the obstetric risks determined by AC-FET exposure. In particular, we inferred a convincing causal relationship between AC-FET and HDP and PE and a probable causal relationship between AC-FET and vPTB, PTB and PPH (Table 1). On this basis, we would like to propose an alternative strategy to the total abandonment of AC-FET in women who ovulate regularly, pending that ongoing research efforts succeed in identifying an endometrial preparation protocol that maintains the 'organizational' advantages of AC-FET without exposing patients to iatrogenic obstetric risks. We suggest avoiding the combination of two or more exposure factors that have a convincing or probable causal relationship with the same adverse outcome or with different outcomes which, if coexisting, could determine the onset of an ominous obstetric syndrome. In other words, our proposal is based on the stratification of obstetric risk in the preconception phase. In recent years, for example, the association between gynecological conditions (i.e. endometriosis, adenomyosis, uterine myomas, polycystic ovary syndrome, cesarean scar, etc.) and obstetric complications has been

**Table 1.** Artificial cycle frozen embryo transfer (AC-FET) and adverse obstetric outcomes: association or causation? A re-analysis of Zaat *et al.*'s findings.

| Outcome | Synthesis of evidence | Quality of evidence (GRADE) | Bradford Hill's criteria | | | | | | Overall judgment about causality |
|---|---|---|---|---|---|---|---|---|---|
| | | | Strength | Consistency | Temporality | Specificity | Plausibility | Coherence | |
| Hypertensive disorders of pregnancy (HDP) | Lower risk in NC-FET pregnancies | Moderate | ** | ** | * | // | * | * | Convincing |
| Pre-eclampsia (PE) | Lower risk in NC-FET pregnancies | Moderate | ** | ** | * | // | * | * | Convincing |
| Placenta previa | Lower risk in NC-FET pregnancies | Moderate | * | // | * | // | // | // | Limited—No conclusion |
| Early pregnancy loss | Lower risk in NC-FET pregnancies | Low | * | // | * | // | // | // | Limited—No conclusion |
| Gestational diabetes mellitus (GDM) | No association | Very low | // | // | * | // | // | // | Substantial effect on risk unlikely |
| Very preterm birth (vPTB) | Lower risk in NC-FET pregnancies | Moderate | * | ** | * | // | * | // | Probable |
| Preterm birth (PTB) | Lower risk in NC-FET pregnancies | Moderate | * | ** | * | // | * | // | Probable |
| Small for gestational age (SGA) | No association | Low | // | // | * | // | // | // | Substantial effect on risk unlikely |
| Low birthweight (LBW) | Lower risk in NC-FET pregnancies | Moderate | * | /* | * | // | // | // | Limited—Suggestive |
| Large for gestational age (LGA) | Lower risk in NC-FET pregnancies | Moderate | * | /* | * | // | // | // | Limited—Suggestive |
| Macrosomia | Lower risk in NC-FET pregnancies | Low | * | * | * | // | // | // | Limited—Suggestive |
| Congenital malformations | No association | Very low | // | // | * | // | // | // | Substantial effect on risk unlikely |
| Neonatal mortality | No association | Very low | // | // | * | // | // | // | Substantial effect on risk unlikely |
| Postpartum hemorrhage (PPH) | Lower risk in NC-FET pregnancies | Very low | ** | * | * | // | * | * | Probable |

NC-FET: natural cycle frozen embryo transfer. Interpretation of the Grading of Recommendations Assessment, Development and Evaluation (GRADE): (i) high: there is high confidence that the true effect lies close to that of the estimate of the effect; (ii) moderate: there is moderate confidence in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; (iii) low: the panel's confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect; and (iv) very low: there is little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect. Strength was defined according to the following criteria: (i) odds ratio (OR) ≤0.6 and upper bound of the 95% confidence interval (CI) ≤0.7: strong association (**); (ii) OR >0.6 and upper bound of the 95% CI <1: moderate association (*); (iii) upper bound of the 95% CI >1: absence of an association (//). Consistency was judged as high (**), moderate (*), or absent (//) on the basis of the following criteria: (i) consistency of findings across studies; (ii) confirmation of the association after pooling of adjusted OR; (iii) the association was independent from luteal phase support (LPS) in NC-FET. The temporality was graded as follows: (i) present (*); (ii) absent (//). The specificity was graded as follows: (i) present (*); (ii) absent (//). The coherence was graded as follows: (i) present (*); (ii) absent (//). The following Bradford Hill's criteria were judged not applicable: biological gradient, experiment and analogy. Causality was judged on the basis of the Continuous Update Project (CUP) of the World Cancer Research Fund's 5 levels of evidence. Specifically: (i) 'Convincing': evidence strong enough to support a judgment of a convincing causal relationship, which justifies making recommendations designed to reduce the risk of cancer. The evidence is robust to be unlikely to be modified in the foreseeable future as new evidence accumulates; (ii) 'Probable': evidence strong enough to support a judgment of a probable causal relationship; (iii) 'Limited—Suggestive': evidence that is too limited to permit a probable or convincing causal judgment but is suggestive of a direction of effect; (iv) 'Limited—No conclusion": evidence is too limited that no firm conclusion can be made. This judgment represents an entry level and is intended to allow any exposure for which there are sufficient data to want consideration, but where insufficient evidence exists to permit a more definitive grading; (v) "Substantial effect on risk unlikely": evidence is strong enough to support a judgment that a particular exposure is unlikely to have a substantial causal relation to an outcome. The evidence should be robust enough to be unlikely to be modified in the foreseeable future as new evidence accumulates. The CUP criteria state that the conclusions of convincing or probable are strong enough to support a recommendation, while evidence judged to fall into either of the limited categories is generally not strong enough to support recommendations.

extensively investigated (Chatzakis *et al.*, 2022; Landman *et al.*, 2022; Lin *et al.*, 2022; Vercellini *et al.*, 2023). For example, the entity of the effect estimate on the one hand and the biological plausibility on the other, allow us to hypothesize a probable causal relationship between severe adenomyosis and the development of PE (Vercellini *et al.*, 2023). In light of the insights provided by the meta-analysis of Zaat *et al.* and our re-analysis of its findings, it seems wise to recommend avoiding AC-FET in women with severe adenomyosis (Zaat *et al.*, 2023). In fact, since the probable pathophysiological mechanisms linking each exposure factor (i.e. adenomyosis and AC-FET) to PE are independent, one could speculate that their effect on the risk of PE is additive. Similarly, deep invasive endometriosis (DIE) was shown to have a probable causal relationship with PTB (Vercellini *et al.*, 2023). Adopting AC-

FET in women with DIE would likely further increase the risk of PTB. Other examples could be given but their description is beyond the scope of this letter. This approach could be applicable not only to AC-FET but to all IVF related factors (freezing/thawing procedure, embryo stage at transfer, embryo biopsy for preimplantation genetic testing etc.) that have been shown to increase specific obstetric or perinatal risks. The IVF journey consists of a series of crossroads in which doctors and biologists are faced with choices to maximize the chances of IVF success. Raising awareness about the impact that each of these choices also has on pregnancy health is of fundamental importance. To this end, it is essential that future research strives to elucidate the causal relationships between each preconceptional or IVF related exposure factor and obstetric and perinatal risks so that clinicians do not become confused by a plethora of associations. The ultimate goal should be the drafting of protocols which, on the one hand, provide for the stratification of preconception obstetric risk and, on the other hand, guide doctors and biologists regarding which choices to make at each crossroads to preserve not only the chances of success of the IVF procedure but also the health of the mother and the fetus.

## Conflict of interest

None declared.

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