Human Reproduction Open, 2023, **2023(3)**, hoad016
https://doi.org/10.1093/hropen/hoad016
Advance Access Publication Date: May 15, 2023
Letter to the Editor

Caution is needed when communicating analyses based on an apple to orange comparison

Sir,

It was with great interest that we read 'The effect of frozen embryo transfer regimen on the association between serum progesterone and live birth: a multi-centre prospective cohort study (ProFET)' by Melo *et al.* (2022). From their data, the authors concluded that overall serum progesterone levels (P4) <7.8 ng/ml are associated with reduced odds of live birth in frozen embryo transfer (FET). Interestingly, the authors previously published a meta-analysis (Melo *et al.*, 2021) based on several cohort studies of HRT-FET cycles using vaginal progesterone for luteal phase support and reporting a higher P4 cut-off <10 ng/ml for the reproductive outcome. Thus, in that analysis, higher serum P4 levels were associated with increased ongoing pregnancy or live birth rates (LBRs). An important question to ask in relation to the newest publication by Melo *et al.* (2022) would be: is this suggested new cut-off of serum P4 of 7.8 ng/ml more accurate than 10 ng/ml, and is this cut-off applicable to all FET protocols?

Reading the publication carefully reveals that the present study was powered to 900 FET cycles; however, only a total of 398 cycles were included in the final analysis. Furthermore, the cohort of FET protocols was very heterogeneous, including HRT-FET, true natural cycle (t-NC), and modified natural cycle (m-NC), in which ovulation is induced with a trigger bolus of hCG. In this context, we have to bear in mind that the FET protocols mentioned are very different in terms of basic endocrinology, first and foremost when considering serum P4. Thus, the natural cycle has a circadian luteal phase progesterone pattern due to the endogenous production of progesterone from the corpus luteum and importantly, in the new Melo *et al.* (2022) study, a huge variation in the type of 'NC FET' protocols was allowed. Thus, different hCG-trigger doses (5000 vs 6500 IE) were used which will definitely have an impact on circulating luteal P4; moreover, in some cycles, no hCG trigger (t-NC) was used and some cycles had vaginal progesterone support whereas others did not. Finally, different dosing and types of vaginal micronized progesterone were used (Cyclogest®, Utrogestan®). Altogether, within a cohort of 45 'NC FET', there might have been as many as nine different combinations; importantly, these differences will invariably result in significant differences in luteal P4 profiles.

Furthermore, in the cohort of HRT-FET cycles, we also learn that important differences were allowed in terms of different vaginal micronized progesterone products, differences in dosing regimen and differences in no use or use of a combination of subcutaneous (s.c.) progesterone (Lubion®), 25 mg once daily or twice daily.

For monitoring, the authors state that blood sampling was performed ~4–6 h after the last administration of exogenous progesterone. Again, the reader might ask, what does 'approximately' mean? One hour, two hours—or more? Timing of luteal phase blood sampling is crucial, especially when considering an exogenous progesterone regimen including s.c. progesterone. Thus, after s.c. injection of water-soluble progesterone, a 10-fold increase in the serum P4 level is seen and, as early as after 1 h, the P4 serum level starts decreasing. As previously reported, the mean P4 level 24 h after administration of 25 mg s.c. progesterone is as low as 5 ng/ml (Sator *et al.*, 2013). This complexity in pharmacokinetics is essential for monitoring of blood sampling.

Although the Melo et al. (2022) study was prospective and P4 levels were blinded for the clinicians, the multiple protocol variations used were entirely owing to clinicans' preference, which may also have influenced the results.

The most interesting finding of the Melo et al. (2022) study is that s.c. progesterone 25 mg twice daily was used as a 'standard HRT-FET' protocol. To our knowledge, this is the first prospective study to report LBR in HRT-FET cycles using only s.c. progesterone. Although the study was not powered for LBR and only 57 FET cycles were included, an LBR of 28% compared to the HRT-FET LBR of 43% could be considered rather low, and a total miscarriage rate of 41% high, indicating that s.c. progesterone as a stand-alone treatment in the dosing described is not sufficient for the HRT-FET cycle.

Regardless of the meticulous statistical analysis and graphic communication in this publication, we challenge the authors as to whether it is good scientific 'evidence' to suggest a new cut-off level of 7.8 ng/ml for more or less all FET protocols, based on the reproductive outcome of 10% of a very heterogeneous cohort, including a total of 398 FET cycles in a study powered to 900 cycles.

As clinicians, we should always critically review dazzling statistics—especially when apples are compared to oranges.

Conflict of interest

The authors have no conflict of interest to disclose.

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