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REVIEW *Infertility*

Frozen embryo transfer: a review on the optimal endometrial preparation and timing

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STUDY QUESTION: What is the optimal endometrial preparation protocol for a frozen embryo transfer (FET)?

SUMMARY ANSWER: Although the optimal endometrial preparation protocol for FET needs further research and is yet to be determined, we propose a standardized timing strategy based on the current available evidence which could assist in the harmonization and comparability of clinic practice and future trials.

WHAT IS KNOWN ALREADY: Amid a continuous increase in the number of FET cycles, determining the optimal endometrial preparation protocol has become paramount to maximize ART success. In current daily practice, different FET preparation methods and timing strategies are used.

STUDY DESIGN, SIZE, DURATION: This is a review of the current literature on FET preparation methods, with special attention to the timing of the embryo transfer.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Literature on the topic was retrieved in PubMed and references from relevant articles were investigated until June 2017.

MAIN RESULTS AND THE ROLE OF CHANCE: The number of high quality randomized controlled trials (RCTs) is scarce and, hence, the evidence for the best protocol for FET is poor. Future research should compare both the pregnancy and neonatal outcomes between HRT and true natural cycle (NC) FET. In terms of embryo transfer timing, we propose to start progesterone intake on the theoretical day of oocyte retrieval in HRT and to perform blastocyst transfer at hCG + 7 or LH + 6 in modified or true NC, respectively.

LIMITATIONS REASONS FOR CAUTION: As only a few high quality RCTs on the optimal preparation for FET are available in the existing literature, no definitive conclusion for benefit of one protocol over the other can be drawn so far.

WIDER IMPLICATIONS OF THE FINDINGS: Caution when using HRT for FET is warranted since the rate of early pregnancy loss is alarmingly high in some reports.

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Introduction

More efficient cryopreservation strategies (i.e. vitrification) (Loutradi *et al.*, 2008) and reassuring safety data (Belva *et al.*, 2008; 2016) have progressively increased the use of frozen embryo transfer (FET) (European IVF-Monitoring Consortium (EIM) *et al.*, 2016), namely beyond cases with a surplus amount of good quality embryos following an elective single embryo transfer policy (Peeraer *et al.*, 2014). The use of an antagonist protocol with agonist triggering followed by a 'freeze-all' strategy and transfer of the embryo(s) in a subsequent FET cycle is a promising option with high live birth rates (Blockeel *et al.*, 2016). Although elective embryo cryopreservation was mainly developed for patients with an increased risk of developing ovarian hyperstimulation syndrome (Devroey *et al.*, 2011), its use has now been also extended to cycles with pre-implantation genetic diagnosis/screening, late-follicular progesterone elevation (Bosch *et al.*, 2010; Roque *et al.*, 2015; Healy *et al.*, 2016) and embryo-endometrial asynchrony (Shapiro *et al.*, 2008). Moreover, there is an ongoing debate whether frozen embryos transferred in a 'more physiologic' non-stimulated endometrium, may not only result in higher pregnancy rates (Shapiro *et al.*, 2011; Roque *et al.*, 2013), but also potentially decrease maternal and neonatal morbidity (Evans *et al.*, 2014; Ishihara *et al.*, 2014).

Materials and Methods

In the following review, we gather the available evidence in search for the best preparation protocol for FET. Literature on the topic was retrieved in PubMed and references from relevant articles were investigated until June 2017.

Results

FET preparation methods

FET preparation methods can largely be divided into artificial and natural cycles (NCs). In the artificial cycle, also referred to as a HRT cycle, endometrial proliferation and follicular growth suppression is achieved by estrogen supplementation. Meanwhile, in the NC, solely menstrual cycle monitoring is performed usually without any pharmacological intervention prior to ovulation.

Hormonal replacement treatment

Although originally developed to allow embryo transfers in recipients of donated oocytes, the HRT protocol has proven to be successful in the general population as well (Younis *et al.*, 1996), thus extending its advantages in terms of minimal monitoring and easy scheduling to those performing IVF overall. However, the universal application of HRT cycles may have potential disadvantages including an increased cost, inconvenience and the potential adverse events associated with estrogen supplementation (e.g. increased thrombotic risk).

Estrogen supplementation. Most HRT protocols empirically opt to supplement estrogens for 2 weeks in an attempt to mimic the NC (Lutjen *et al.*, 1984). However, it seems that such an extended period may be unnecessary and that 5–7 days may suffice for adequate endometrial proliferation (Navot *et al.*, 1986). Limiting the length of the estrogen supplementation would be beneficial in terms of cost and time to pregnancy and deserves further attention in upcoming studies. Caution, however, is warranted, given that a higher miscarriage rate with shorter estrogen supplementation has also been previously reported (Borini *et al.*, 2001). Conversely, if necessary, estrogen supplementation may also be safely prolonged if necessary without compromising pregnancy outcome (Soares *et al.*, 2005).

Estrogens may be administered orally, vaginally and parentally (transdermal route) and both natural as well as synthetic estrogens may be used (Scott *et al.*, 1991b). A meta-analysis concluded that the type of estrogen supplementation and route of administration had no effect on the success rates of FETs (Glujovsky *et al.*, 2010). The conversion between different supplementation methods may be estimated as follows: 0.75 mg of micronised estradiol (oral administration) = 1.25 g of estradiol gel (transdermal administration) = 1 mg of estradiol valerate (oral or vaginal adminstration). The standard dose of estradiol valerate is 6 mg daily (Cobo *et al.*, 2012), although different step up protocols—mimicking the rising estradiol levels of a NC—are also frequently used (Soares *et al.*, 2005; Escribá *et al.*, 2006; van de Vijver *et al.*, 2014).

Exogenous mild ovarian stimulation instead of direct estrogen supplementation has been proposed aiming to increase the circulation of serum estrogen and potentially enhance endometrial receptivity. However, a recent systematic review concluded that, when compared to NC, ovarian stimulation with gonadotropins or clomiphene citrate did not seem to enhance live birth pregnancy rates (Yarali *et al.*, 2016). Interestingly, when compared to HRT, gonadotropins or letrozole ovarian stimulation did seem to have a slightly increased chance for live birth. However, until well-designed prospective studies are performed, no definitive recommendation on the use of ovarian stimulation during FET can be made.

Monitoring during estrogen supplementation. In daily clinical practice, an ultrasound scan is usually planned following an initial period of estrogen priming in order to measure endometrial thickness and exclude the presence of a pre-ovulatory follicle, corpus luteum or luteinized endometrium prior to starting progesterone supplementation. The optimal endometrial thickness in HRT FET cycles has been described to be between 9 and 14 mm (El-Toukhy *et al.*, 2008). Conversely, given that a previous meta-analysis has associated endometrial thickness $\leq$ 7 mm in fresh IVF cycles with a lower chance of pregnancy, this cut-off value is generally extrapolated to FET as well; however, the actual value of this arbitrary cut-off and whether the same limit can be extrapolated to frozen cycles requires further research (Dain *et al.*, 2013; Kasius *et al.*, 2014).

There is limited information available regarding the need for endocrine monitoring during HRT. Specifically, late-follicular serum estradiol and luteinizing hormone (LH) do not seem to predict outcome (Remohi *et al.*, 1997; Banz *et al.*, 2002; Griesinger *et al.*, 2007; Niu *et al.*, 2008; Bocca *et al.*, 2015). Serum progesterone assessments may be used to detect escape ovulation, an event which can be encountered in 1.9–7.4% of HRT FET cycles without pituitary suppression (Dal Prato *et al.*, 2002; van de Vijver *et al.*, 2014). However, given the low incidence, it is questionable whether this measurement significantly improves pregnancy outcome, definitely when additional preventive measures are taken to avoid follicular growth and escape ovulation (e.g. high dose of estrogen supplementation from Day 1 of the cycle onwards).

GnRH agonist. Besides the administration of estrogen, a GnRH agonist can be added to a HRT protocol in order to prevent spontaneous ovulation (Keltz *et al.*, 1995). In one randomized controlled trial (RCT), the use of such an approach was associated with increased clinical pregnancy and live birth rates, mainly due to lower cycle cancellation rates (El-Toukhy *et al.*, 2004). However, endocrine cycle monitoring was not performed in that study, and the incidence of premature ovulation was not reported. The results of this trial are also in contradiction with those of subsequent systematic reviews and meta-analyses, which failed to demonstrate any benefit in terms of clinical pregnancy and cancellation rates (Ghobara and Vandekerckhove, 2008; Glujovsky *et al.*, 2010). More recently, another retrospective study also failed to show any benefit of the use of a GnRH agonist (van de Vijver *et al.*, 2014). Conversely, HRT FET cycles without GnRH agonist co-treatment seem to be more patient-friendly given the avoidance of the cost and potential side effects associated with these drugs.

Progesterone supplementation. Once the proliferation of the endometrium with the administration of estrogens is considered sufficient, progesterone is initiated to promote the final phase of endometrial preparation prior to embryo transfer. An additional injection of hCG on the day of progesterone initiation showed no better implantation or pregnancy rates (Ben-Meir *et al.*, 2010). Regarding progesterone supplementation itself, there is little agreement on the ideal route of administration and dose. Often, micronized progesterone is administered vaginally (Bourgain *et al.*, 1990). When compared to intramuscular (IM) injections, patients seem to prefer the vaginal route owing to its quick, easy and painless administration (Levine, 2000). However, there is no RCT comparing IM and vaginal routes in HRT FET cycles. Retrospective data are conflicting, being in favor of the IM route (Haddad *et al.*, 2007; Kaser *et al.*, 2012) or showing no significant differences in terms of outcome (Shapiro *et al.*, 2014). A recent double-blinded placebo-controlled RCT demonstrated non-inferiority and a similar safety profile for the oral administration of dydrogesterone in fresh cycles (Tournaye *et al.*, 2017). However, more data are needed to confirm the safety and efficacy of oral dydrogesterone in HRT FET. As for the optimal progesterone dose specifically in HRT FET cycles, one retrospective study concluded that doubling the dose of vaginal progesterone gel in patients with oligomenorrhoea significantly increased live birth rates (Alsbjerg *et al.*, 2013).

The use of measuring serum progesterone during the luteal phase in HRT FET cycles requires further investigation as well. The currently available results are contradictory as progesterone levels >20 ng/ml (possibly due to an escape ovulation and subsequent embryo-endometrial asynchrony) on the day of transfer have been associated with decreased ongoing pregnancy and live birth rates (Kofinas *et al.*, 2015), while an optimal mid-luteal progesterone range between 22 and 31 ng/ml has also been proposed (Yovich *et al.*, 2015). The administration route and dose also needs to be taken into account when performing such endocrine monitoring. Furthermore, another potential confounding factor is intercourse during a FET cycle, since it has been shown that it significantly reduces serum progesterone levels in women administering vaginal progesterone gel (Merriam *et al.*, 2015).

No consensus has been reached yet on when to stop progesterone administration following a positive pregnancy test in HRT FET. The estimated onset of placental steroidogenesis, the so-called luteoplacental shift, occurs during the fifth gestational week (Scott *et al.*, 1991a). A meta-analysis has demonstrated that, following a fresh embryo transfer, progesterone can be discontinued once a positive pregnancy test is detected (Liu *et al.*, 2012). However in HRT FET cycles, as no corpus luteum— and, hence, no endogenous progesterone production—is present, the best moment remains to be elucidated.

Natural cycle

In a NC FET, there is no medical intervention, except of endocrine and ultrasound monitoring during the proliferative phase, to schedule the transfer when the endometrium is synchronized to the developmental stage of the embryo. Although the advantage is the absence of estrogen supplementation, this protocol entails more frequent visits to the clinic, less cycle control and flexibility and holds a higher risk of cycle cancellation [up to 6% (Sathanandan *et al.*, 1991)].

Proliferative phase monitoring. The starting point to assess embryo-endometrial synchronization is the ovulation of the dominant follicle, which in a NC can either be triggered exogenously (i.e. modified NC, in which ovulation is triggered by hCG as soon as a dominant follicle of e.g. >16 mm is observed) or by serial blood (or, albeit less accurately, urine) sampling until a LH peak is observed (i.e. true NC, in which ovulation occurs spontaneously). Although the serum hormone levels in such cases are often exhaustively assessed (Casper *et al.*, 2016), the role of such endocrine monitoring in addition to the usual ultrasound monitoring is a subject of much debate in both true and modified NC FETs (Groenewoud *et al.*, 2012, 2017; Lee *et al.*, 2014). Furthermore, the definition of what constitutes an LH surge is not unanimous. Historically, an LH surge has been described as an increase of the level of LH beyond 180% of the mean level observed in the previous 24 h (Frydman *et al.*, 1982). In a clinical setting, however, varying definitions are used, including a concentration of 180% above the latest serum value available in that patient with a continued rise thereafter (Testart *et al.*, 1981) to a level of 10 IU/l or more (Groenewoud *et al.*, 2017).

Regarding endometrial thickness, the optimal threshold for NC FET remains unknown and the extrapolation of findings in fresh and HRT FET cycles should also be approached with caution in this case given the lack of data.

Spontaneous versus triggered ovulation. Two small RCTs revealed conflicting results: while the first (Weissman *et al.*, 2011) did not find any significant differences between spontaneous and exogenously-triggered ovulation cycles, another (Fatemi *et al.*, 2010) was interrupted prematurely due to the fact that an interim analysis revealed remarkably lower pregnancy rates in women who were administered hCG (14.3% versus 31.4%, respectively). One of the posited reasons for this difference was that the research groups had considered different timings to perform the embryo transfer (specifically, a 1-day difference between both studies). Second, it is possible that in the prematurely interrupted study there could have been a higher embryo-endometrial asynchrony in the modified NC study group as FET timing was the same for both arms, despite known differences in the timing of spontaneous versus triggered ovulation (Kosmas *et al.*, 2007). Third, some women from the modified NC group in this same study already had an LH rise on the day of hCG administration which

was associated with significantly lower pregnancy rates (suspected to be because of higher grade of embryo-endometrial asynchrony), while serum progesterone >1 ng/ml was an exclusion criterion in the study by Weissman *et al.* Finally, luteal phase support (LPS) was given only in the RCT performed by Weissman *et al.*

Three retrospective studies comparing true versus modified NC failed to demonstrate significant differences in clinical outcomes (Weissman *et al.*, 2009; Chang *et al.*, 2011; Tomás *et al.*, 2012), however a recent large retrospective analysis did show a significant difference in clinical pregnancy rate (CPR) in favor of the true NC FET (without LPS) versus the modified NC FET (with LPS) even after adapting the transfer policy to the type of ovulation trigger and excluding patients that administered hCG despite a LH surge (46.9% versus 29.7%, $P < 0.001$) (Montagut *et al.*, 2016). Thus, until further prospective studies comparing true with modified NC are performed, the question on what seems the best approach remains unanswered.

Progesterone supplementation. The prevalence of a luteal phase defect in NCs in normo-ovulatory subfertility patients has been historically described to be around 8% (Rosenberg *et al.*, 1980), with mid-luteal serum progesterone levels <10 ng/ml being considered to reflect a NC luteal phase defect (Jordan *et al.*, 1994).

The use of LPS in true NC FET is supported by one RCT (Bjuresten *et al.*, 2011) where micronized vaginal progesterone (MVP) was initiated in the evening after FET. Our retrospective analysis (Montagut *et al.*, 2016) did not show a significant difference in CPR when comparing true NC FET with or without MVP; on the contrary, there was a trend favouring one not to supplement (CPR 46.9% versus 39.9%). Here, however, MVP was started sooner, immediately on the day after the LH surge. Hence, the discrepancy between the studies might reflect the importance of the correct timing to start LPS. Another retrospective study investigating true NC FET LPS by two IM injections of hCG (the day of FET and 6 days later) failed to show any difference in outcome (Lee *et al.*, 2013).

For modified NC FET, both prospective (Eftekhar *et al.*, 2013) and retrospective (Kyrou *et al.*, 2010) studies failed to show any difference in terms of pregnancy outcome with or without LPS. Due to prolonged half-life of hCG used as trigger, it makes biological sense that no LPS may be needed, although not all researchers agree (Kim *et al.*, 2014).

Overall, the moment to start LPS in a NC FET is unclear although one may postulate that immediately after the LH surge or hCG trigger may be too soon and affect the window of implantation (WOI). Until further data are accrued on this subject it seems likely that different protocols will continue to be used in daily practice (Weissman *et al.*, 2011; Tomás *et al.*, 2012).

HRT or NC?

Retrospective data have left physicians with conflicting information in terms of clinical outcome (Ghobara and Vandekerckhove, 2008; Givens *et al.*, 2009; Chang *et al.*, 2011; Groenewoud *et al.*, 2013; Guan *et al.*, 2016). Recently, a large, multi-center, non-inferiority trial studying modified NC versus HRT has failed to show any significant difference in live birth, clinical or ongoing pregnancy rates (Groenewoud *et al.*, 2016). Furthermore, the costs of both treatment modalities were comparable. However, this study did not assess the potential benefit of FET performed without exogenous ovulation triggering and concerns were raised due to the overall low success rate reported and the high miscarriage rates (Hreinsson *et al.*, 2016). A previous retrospective analysis has shown a higher miscarriage rate for HRT compared to NC FET, although this could be related to the higher proportion of polycystic ovary syndrome patients in the HRT group (Tomás *et al.*, 2012). Additionally, when comparing HRT FET to fresh embryo transfer, a 1.7-fold higher miscarriage rate has also been described for hormonal substitution FET *per se* (Veleva *et al.*, 2008) and, in cases of repeated implantation failure endometrial transcriptome analysis favored NC over HRT (Altmäe *et al.*, 2016). Current caution and further research is needed; a RCT comparing true NC versus HRT FET in an unbiased population is warranted.

FET timing

The synchronous interaction between a competent embryo and a receptive endometrium is a complex molecular process indispensable for successful implantation (Tabibzadeh, 1998). It is generally considered that once progesterone levels reach a critical threshold, they set into motion a well-timed and orderly secretory transformation of the endometrium leading to receptivity (Franasiak *et al.*, 2016). This receptiveness for blastocyst attachment only occurs for a short period, the WOI (Psychoyos, 1973; Bergh and Navot, 1992). Decidualization, the secretory transformation that the endometrial stromal compartment undergoes to accommodate pregnancy, plays an important role in receptivity as it is thought to contribute to the active selection of embryos attempting implantation (Brosens *et al.*, 2014). Hence, FET timing should assure that the blastocyst seeking implantation meets the optimal receptive/selective endometrial stage during the WOI. Many efforts have been made to identify biomarkers of endometrial receptivity (Coutifaris *et al.*, 2004; Díaz-Gimeno *et al.*, 2011; Edgell *et al.*, 2013), but, so far, no clinically RCT validated test is available in daily practice.

Hormonal replacement treatment

The optimal duration of exposure to progesterone prior to embryo transfer has remained an elusive topic since the start of ART (Nawroth and Ludwig, 2005). When progesterone supplementation in HRT cycles is initiated 3 days before the cleavage embryo transfer, excellent pregnancy rates of up to 40.5% occur (Givens *et al.*, 2009). A limited amount of evidence indicates that even a very short progesterone exposure may suffice to induce endometrial receptivity (Imbar and Hurwitz, 2004; Theodorou and Forman, 2012). Conversely, a study conducted in oocyte recipients showed a higher biochemical pregnancy rate when progesterone supplementation was longer (i.e. transfer of a Day 3 embryo on the 5th day of progesterone supplementation) (Escribá *et al.*, 2006). In line with this, it has been suggested that the risk of early pregnancy loss increases when implantation takes place later in the WOI (Wilcox *et al.*, 1999). A Cochrane Database Review concluded that starting progesterone at a time equivalent to the day of or the day after oocyte retrieval (OR) results in a significantly higher pregnancy rate than if progesterone is initiated a day earlier than the day equivalent to OR (Glujovsky *et al.*, 2010). A recent RCT compared the outcomes of blastocyst transfer with either 5 or 7 days of progesterone supplementation and CPRs once more tended to be in favor of the shorter protocol, although not statistically significant (32.5% versus 27.6%) (van de Vijver *et al.*, 2017). On the other hand, transferring Day 4 embryos on the third day of progesterone supplementation (a time being equivalent to 2 days after OR) was also deleterious (van de Vijver *et al.*, 2016).

Specifically, a higher risk of early pregnancy loss was seen, possibly caused by embryo-endometrial asynchrony or by an insufficient decidualization associated with only 3 days of progesterone administration. Another hypothesis is that, due to a later timing of the WOI, delayed embryos may have a higher chance of encountering a receptive endometrium, allowing them to implant but then being at increased risk for early pregnancy loss.

Taken together, it seems that the starting day of progesterone intake is optimal when equal to the theoretical day of OR or 1 day later (Fig. 1). Given that the WOI is limited in time, this detection of an optimal period is unsurprising and easily understandable; implantation is possible in a quite broad window, but only optimal in a narrower timeframe (Franasiak *et al.*, 2016). Currently, most cleavage stage embryos are transferred around the 4th day of progesterone supplementation, whereas blastocysts are usually transferred on the 6th day of progesterone supplementation. This presumptive embryo transfer timing is in parallel with the timing of fresh embryo transfer after OR: the day of starting progesterone supplementation (considered as P + 0) is set equal to the theoretical day of OR, which is indeed also Day 0 from an embryonic point of view. This should be the preferred terminology as it emphasizes the synchronicity between endometrium and embryo. In a time when embryo transfer may soon become personalized according to a prior diagnostic intervention (e.g. Endometrial Receptivity Array, ERA®, Igenomix) (Díaz-Gimeno *et al.*, 2011), the use of a standardized nomenclature is of utmost importance. Specifically, in repeated implantation failure patients, the WOI is suspected to be narrow and/or displaced (mostly delayed) (Ruiz-Alonso *et al.*, 2013). Meanwhile, even in the general population, delayed endometrial development has been described in up to 25% of the population (Murray *et al.*, 2004) and an increase in pregnancy rates associated with specific histological endometrial dating patterns and corresponding adjustments in progesterone exposure has been shown (Gomaa *et al.*, 2015).

Natural cycle

In a NC, the WOI is posited to open 6 days after the postovulatory progesterone surge and thought to last ~2–4 days (LH + 7 to LH + 11) (Navot *et al.*, 1991). When using the LH surge to plan embryo transfer one must take into account that the LH surge can occur over a period of 30 h (Acosta *et al.*, 2000). Progesterone rises slightly to 1–3 ng/ml even 12 h to 3 days prior to ovulation, due to the LH-stimulated production by the peripheral granulosa cells (Hoff *et al.*, 1983), with a steep increase in production following ovulation (3–10 ng/ml) due to production by the corpus luteum. The physiological and clinical importance of the pre-ovulatory progesterone elevation is yet to be determined, but is likely to contribute to the induction of the WOI in a NC. However, an accurate mirroring of this finely tuned and tightly regulated molecular system is probably difficult to reproduce artificially and one should acknowledge that all interventions might change the opening, closing, length and functionality of the WOI.

A difference in the timing of FET in true versus modified NC could be considered, as ovulation occurs 36–48 h after hCG administration but varies from 24 to 56 h after a spontaneous LH surge (Kosmas

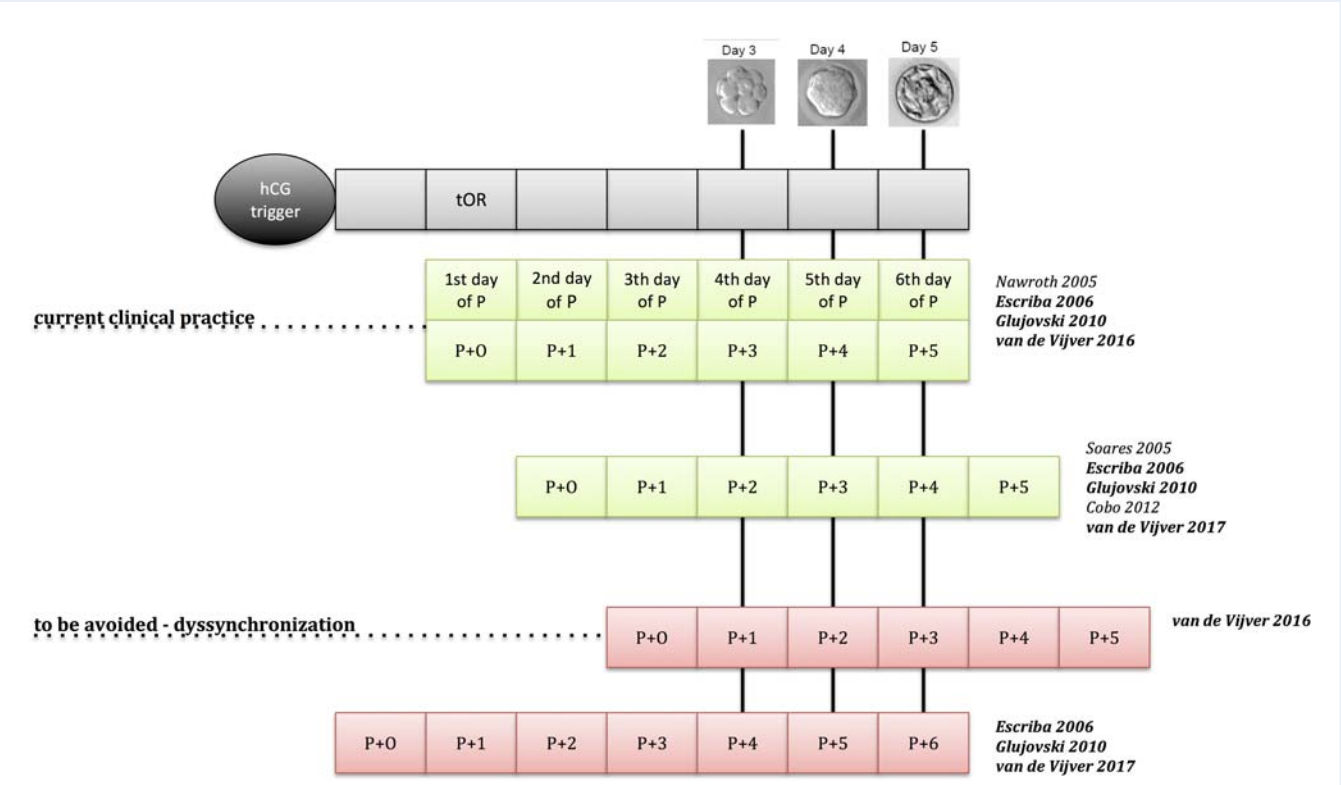

Figure 1 Embryo transfer timing for HRT preparation. tOR, theoretical oocyte retrieval, P, progesterone. In bold: studies with actual comparison of different embryo transfer days.

et al., 2007). For intra-uterine insemination, it has been shown that pregnancy rates are higher when it was performed 36–42 h after hCG trigger, but 18–24 h after spontaneous LH surge (Fuh et al., 1997; Robb et al., 2004). One could draw the parallel to FET and transfer 1-day earlier when a spontaneous LH surge is detected in the serum compared to when ovulation is triggered with hCG. When using urinary LH measurement, this difference in timing might not be beneficial, since a 1-day delay for the detection of peak hormone levels in the urine has been described (Cekan et al., 1986).

We suggest not to administer hCG when a spontaneous LH surge is detected, given the previously noted potential association with a detrimental outcome (Fatemi et al., 2010), even though it has not been confirmed in a recent *post hoc* analysis of the ANTARCTICA trial (Groenewoud et al., 2017). We hypothesize that hCG trigger, as well as additional LPS may impact on the natural course of the endometrium towards receptivity and might cause a shift in the WOI, leading to a more pronounced embryo-endometrial asynchrony. Further research is needed to test this hypothesis and to clearly state what should be the preferred policy in clinical practice.

FET timing proposal

We have observed that in studies assessing the optimal preparation for FET, embryo transfer timing is often described vaguely or confusingly. However, when there was no optimal synchronization, incorrect conclusions on how to best prepare FET could be drawn. We propose the following FET timing strategy and terminology, which could assist in the harmonization and comparability of clinical practice and future trials (Fig. 2):

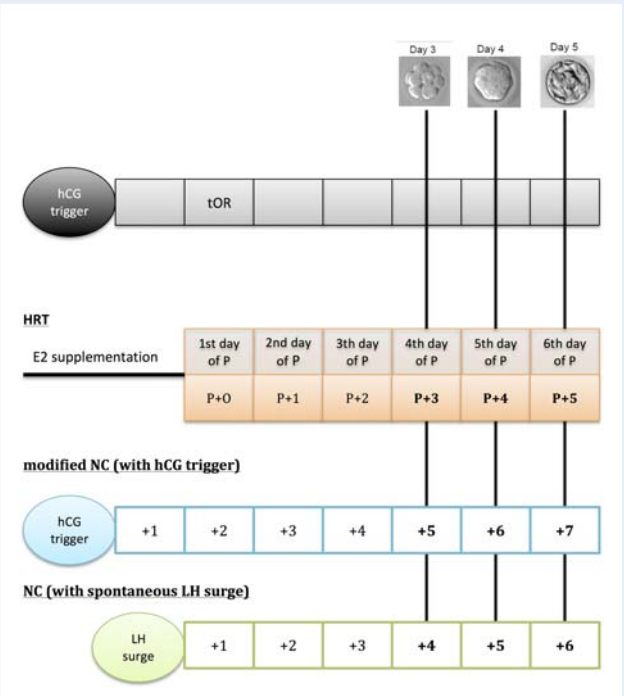

Figure 2 Clinical practice proposal for embryo transfer timing in the different preparation methods. tOR, theoretical oocyte retrieval, E2, estradiol, P, progesterone, NC, natural cycle.

– *In HRT*:
On day (embryonic age + 1) of progesterone administration, annotated as P+ embryonic age (e.g. a Day 5 embryo on the 6th day of progesterone administration, annotated as P + 5).
– *In a modified NC (with hCG trigger)*:
On day (embryonic age + 2) after hCG injection (e.g. a Day 5 embryo on hCG + 7).
– *In a true NC (with spontaneous LH surge)*:
On day (embryonic age + 1) after LH surge (e.g. a Day 5 embryo on LH + 6).

Specific attention is warranted in situations where embryo thawing is followed by further *in vitro* culture and embryonic development prior to transfer. In such cases, it is likely better to take into account the expected embryonic stage at the moment of transfer instead of the stage in which the embryo was cryopreserved (Cercas et al., 2012; Jin et al., 2013; van de Vijver et al., 2016). No studies have investigated whether the timing of FET should be different for embryos cryopreserved by slow-freezing or vitrification. However, an impact has been described of the method of freezing on post-thaw embryo development and metabolism (Balaban et al., 2008; Cercas et al., 2012) and further research into the potential clinical effects of such differences might optimize embryo-endometrial synchrony.

Conclusion and future perspectives

Although FET is increasingly used for multiple indications, the optimal preparation protocol is yet to be determined. At the basic research level, the evidence points toward the NC being superior to HRT. Hence, future research should compare both the pregnancy and neonatal outcomes between HRT and true NC FET. Furthermore, caution when using HRT is warranted since the rate of early pregnancy loss is alarmingly high in some reports.

In terms of embryo transfer timing, we propose to start progesterone intake on the theoretical day of oocyte retrieval in HRT and to perform blastocyst transfer at hCG + 7 or LH + 6 in modified or true NC, respectively. As individual timing of the WOI becomes increasingly substantiated by diagnostics tools, subsequent time corrections might offer further opportunities to increase FET success rates.

Authors' roles

S.M. wrote the manuscript. S.S-.R. participated in the writing of the manuscript. A.V.D.V., A.R., L.V.L. and H.T. contributed to the interpretation and editing of the manuscript. C.B. is responsible for the concept and final revision of the manuscript.

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