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HOW TO PREPARE THE ENDOMETRIUM FOR FROZEN EMBRYO TRANSFER

By

DR. ASHA JENKINS

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EDITOR

Dr.Asha Jenkins M.S (O&G) DNB (O&G) MRM (UK)
(Government Head Quarters Hospital,Thuckalay)
Svasti Fertility Centre (Consultant)
Asha Hospital Campus,Thuckalay
Vijayakumar Hospital (Consultant)
Swamiyarmadam.

Introduction:

Frozen-thawed (FT) embryo transfer is a procedure used for the storage and transfer of excess embryos obtained during in vitro fertilization (IVF)– intracytoplasmic sperm injection (ICSI) cycles. In recent years, improvements in laboratory conditions and limitations on the number of embryos to be transferred have led to a progressive increase in FT embryo transfer cycles. Another preferred practice to prevent multiple pregnancies in IVF cycles is to transfer a single embryo and freeze all surplus embryos. However, the best solution for endometrial preparation in these cycles is still a matter of debate.

The advancements in cryopreservation techniques and use of vitrification have allowed for increased success rates when using frozen embryos. Woman who have a good ovarian reserve and are under 35 often times have embryos that can be frozen. After a woman turns 35, her chance of having embryos left for cryopreservation decrease dramatically. Once an embryo has been cryopreserved it can be stored indefinitely.

Frozen-thawed embryo transfer prevents embryo waste and increases the probability of pregnancy in a single stimulated cycle. Protocols applied in FT cycles aim for endometrial preparation only and are therefore simpler than complicated protocols that aim to develop many follicles. As the treatment for sub fertility increases, so does the importance of FT embryo transfer; however, there is no consensus about which method is the best.

Pregnancy rates following FT embryo transfer have been found to be higher than that following fresh embryo transfer. Further, FT embryo transfer increases the cumulative pregnancy rate and decreases the cost; in addition, it is easy to perform and can be applied in a shorter time duration when compared to repetitive fresh embryo transfers. Therefore, studies have concentrated on factors affecting the success rate of FT embryo transfer cycles.

Various cycle protocols are used for the preparation of the endometrium in an FT embryo transfer cycle. In one of these procedures, the transfer time is determined either by the natural course of a cycle
[i.e., in an ovulatory patient exhibiting a natural (spontaneous) cycle] or by inducing ovulation during the course of a natural cycle.
The second procedure involves the artificial preparation of the endometrium through the administration of exogenous estrogen and progesterone, which can be performed with or without the association of a gonadotropin releasing hormone agonist. In the third procedure, the cycle is stimulated by gonadotropins and ovulation is induced by recombinant-human chorionic gonadotropin (r-Hcg) or HCG; however, this practice is no longer applied.

A Frozen Embryo Transfer Can Occur:

- After a successful fresh transfer when a woman is trying to conceive her next child years later
- After an unsuccessful fresh transfer, anywhere from months to years later when the woman is ready to try again
- When the couple decides to undergo genetic testing on their embryos, which results in the need for cryopreservation of the tested embryos
- When medically a fresh transfer is not in the best interest of the patient
- 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

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:

The HRT protocol has proven to be successful in the general population as well 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

Estrogen Supplementation:

Most HRT protocols empirically opt to supplement estrogens for 2 weeks in an attempt to mimic the NC. Suffice However; it seems that such an extended period may be unnecessary and that 5-7 days may for adequate endometrial proliferation. 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. Conversely, if necessary, estrogen supplementation may also be safely prolonged if necessary, without compromising pregnancy outcome.

Estrogens may be administered orally, vaginally and parentally (transdermal route) and both natural as well as synthetic estrogens may be used. A meta- analysis concluded that the type of estrogen supplementation and route of administration had no effect on the success rates of FETs. The conversion between different supplementation methods may be estimated as follows:

0.75 mg of micronisedestradiol (oral administration)
= 1.25 g of estradiol gel (transdermal administration)
= 1 mg of estradiolvalerate (oral or vaginal administration).

The standard dose of estradiolvalerate is 6 mg daily. although different step up protocols— mimicking the rising estradiol levels of a NC—are also frequently used. 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.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. Conversely, given that a previous meta-analysis has associated endometrial thickness < 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.

GnRH Agonist:

Besides the administration of estrogen, a Gn RH agonist can be added to a HRT protocol in order to prevent spontaneous ovulation. 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. 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. More recently, another retrospective study also failed to show any benefit of the use of a Gn RH agonist. Conversely, HRT FET cycles without Gn RH 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 h CG on the day of progesterone initiation showed no better implantation or pregnancy rates. Regarding progesterone supplementation itself, there is little agreement on the ideal route of administration and dose. Often, micronized progesterone is administered vaginally. When compared to intra-muscular (IM) injections, patients seem to prefer the vaginal route owing to its quick, easy and painless administration.

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 or showing no significant differences in terms of outcome. A recent double-blinded placebo-controlled RCT demonstrated non-inferiority and a similar safety profile for the oral administration of dydrogesterone in fresh cycles. 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.

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, while an optimal mid-luteal progesterone range between

22 and 31 ng/ ml has also been proposed. The administration route and dose also need 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.

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.

A meta-analysis has demonstrated that, following a fresh embryo transfer, progesterone can be discontinued once a positive pregnancy test is detected. However, in HRT FET cycles, as no corpus luteum and, hence, no endogenous progesterone production—ispresent,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%.

FET Timing:

The synchronous interaction between a competent embryo and a receptive endometrium is a complex molecular process indispensable for successful implantation. 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. This receptiveness for blast ocyst attachment only occurs for a short period, the WOI. 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. 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, but, so far, no clinically RCT validated test is available in daily practice.

Hormonal Replacement Treatment:

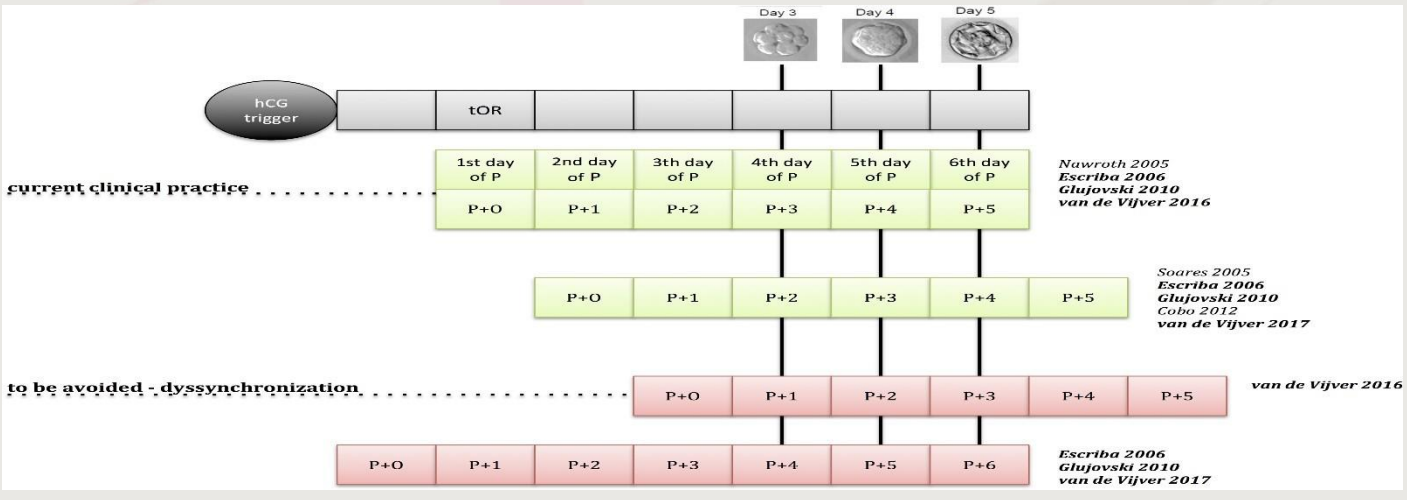
The optimal duration of exposure to progesterone prior to embryo transfer has remained an elusive topic since the start of ART. When progesterone supplementation in HRT cycles is initiated 3 days before the cleavage embryo transfer, excellent pregnancy rates of up to 40.5% occur. A limited amount of evidence indicates that even a very short progesterone exposure may suffice to induce endometrial receptivity. 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).

In line with this, it has been suggested that the risk of early pregnancy loss increases when implantation takes place later in the WOI. 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 r ate than if progesterone is initiated a day earlier than the day equivalent to OR. 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 favour of the shorter protocol, although not statistically significant (32.5% versus 27.6%). 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. 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. 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), 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). Meanwhile, even in the general population, delayed endometrial development has been described in up to 25% of the population and an increase in pregnancy rates associated with specific histological endometrial dating patterns and corresponding adjustments in progesterone exposure has been shown.

Figure 1 Embryo transfer timing for HRT preparation. TOR, theoretical oocyte retrieval, P, progesterone...



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). 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. 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, 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 h CG administration but varies from 24 to 56 h after a spontaneous LH surge. 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. One could draw the parallel to FET and transfer 1-day earlier when a Spontaneous LH surge is detected in theserum 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.

We suggest not to administer hCG when a spontaneous LH surge is detected, given the previously noted potential association with a detrimental outcome, even though it has not been confirmed in a recent post hoc analysis of the ANTARCTICA trial 0.

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):

- 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 h CG trigger):

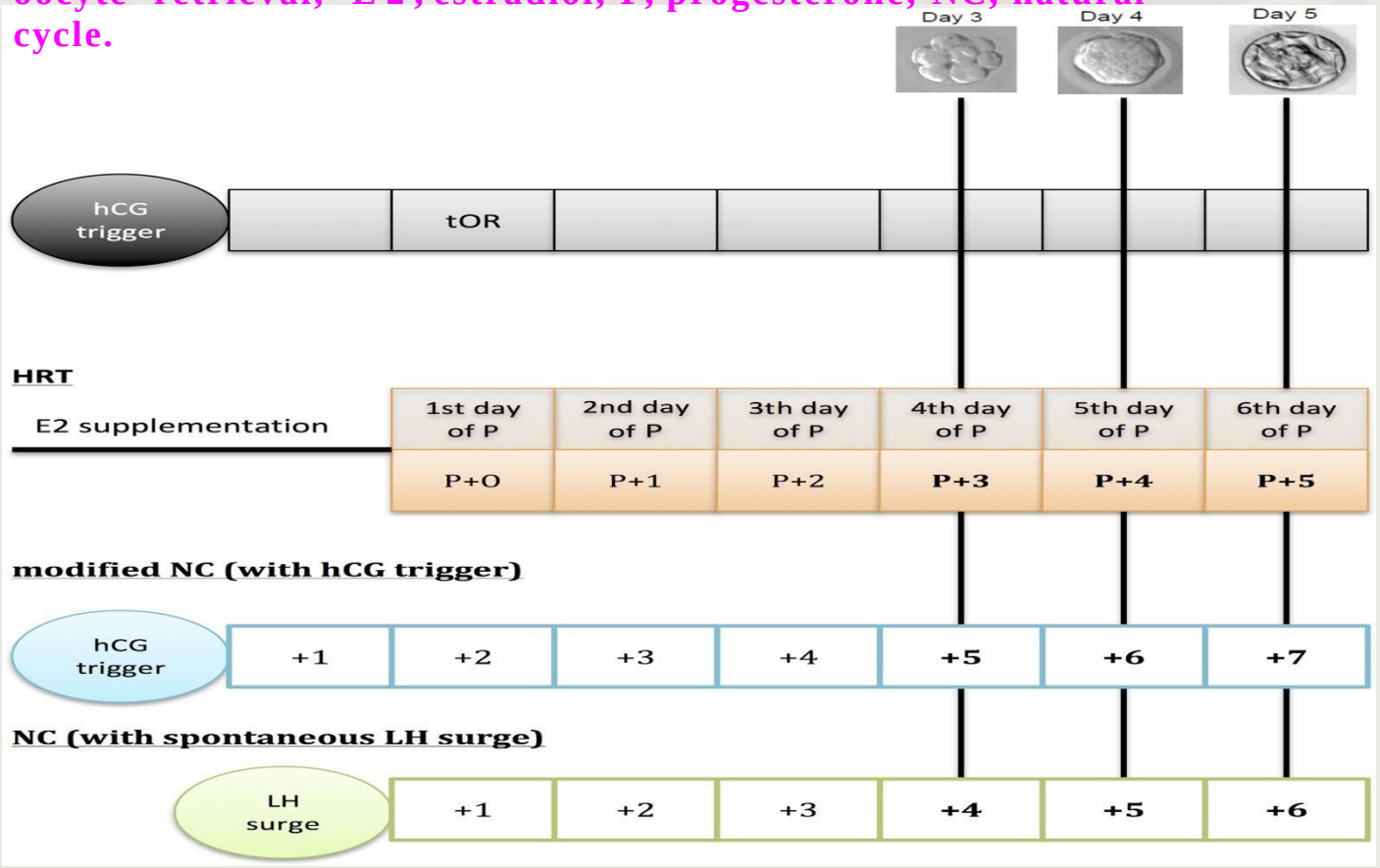
- On day (embryonic age + 2) after h CG injection

(e.g. a Day 5 embryo on hCG + 7).

- —In a t rue NC (with spontaneous LH surge): o On day (embryonic age + 1) after LH surge

(e.g. a Day 5 embryo on LH + 6).

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



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. 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 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.

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