Optimal endometrial preparation for frozen embryo transfer cycles: window of implantation and progesterone support

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With significant improvements in cryopreservation technology (vitrification) the number of frozen ET IVF cycles is increasing and may soon surpass in numbers and success rates those of fresh stimulated IVF cycles. Increasing numbers of elective single ETs are also resulting in more frozen embryos (blastocysts) available for subsequent frozen ET cycles. Optimal endometrial preparation and identification of the receptive window for ET in frozen ET cycles thus assumes utmost importance for insuring the best frozen ET outcomes. Reliable data are essential for defining the optimal endometrial preparation protocols with accurate determination of the implantation window in frozen ET cycles. (Fertil Steril® 2016;105: 867–72. ©2016 by American Society for Reproductive Medicine.)

Key Words: Window of implantation, frozen embryo transfer, endometrial preparation, progesterone support, endometrial receptivity

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WINDOW OF IMPLANTATION: POTENTIAL FOR PERSONALIZED ET

For a human pregnancy to occur, a normal embryo must implant in the endometrium and for this to happen the endometrium must be in a receptive state. In humans, the "window of implantation," the time when the endometrium is most able to support trophoblast-endometrial interactions, is thought to occur during a short period of time around days 22–24 of an idealized 28-day cycle (1).

The endometrium becomes receptive as a result of a series of timed hormonal events during the menstrual cycle. Estrogen (E) stimulates endometrial proliferation and induces progesterone (P) receptors (2). The exposure of the endometrium to P after ovulation initiates morphological and functional alterations that result in the change from a proliferative to a secretory endometrium. The epithelial glands and vasculature continue to grow and become spiral, whereas the endometrial thickness is relatively unchanged, resulting in a denser endometrium. The morphological changes observed on histology for each specific day after ovulation were described by Noyes and his colleagues in 1975 (3) and established the classic endometrial dating paradigm that for the past 6 decades served as the gold standard for clinical evaluation of luteal function.

Besides the histologic changes associated with endometrial receptivity there are multiple molecular and protein alterations that may affect implantation. Around the window of implantation both E receptor (ER) and P receptor (PR) are down-regulated (2). Bruce Lessey et al. (4) were one of the first to show that a number of specific protein and biochemical markers of receptivity are present during the window of implantation. Since then, there have been many reviews of potential markers of implantation without convincing data for clinical utility. Other receptivity tests based on molecular markers have since been developed (5) and most recently microarrays for hundreds of gene expression alterations have been used

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to demarcate the window of implantation (6), with the current status of clinical application having being reviewed by Carlos Simon in this series.

Cryopreservation of human oocytes and embryos has played an increasing role in IVF since the development and refinement of vitrification techniques. In the past, frozen thawed embryo transfers (FET) were associated with lower pregnancy rates (PRs) compared with fresh transfers likely because of less than optimal embryo survival after slow freezing. With improved survival of embryos after vitrification, embryos are now increasingly cryopreserved to facilitate elective single ET and segmentation or "freeze-all" protocols are used to prevent the occurrence of secondary ovarian hyperstimulation syndrome (OHSS) (7). Additional common reasons for freezing all embryos include preimplantation genetic screening/preimplantation genetic diagnosis, premature P rise, and patient or laboratory preference. Other issues, such as possibly superior results compared with fresh ET and the presence of fluid in the endometrial cavity at the time of transfer, are more controversial and need more data. As a result, endometrial preparation to replace warmed embryos so that they can implant at the appropriate time has received much more attention. Unlike fresh ET cycles, vitrified/warmed ET allows adjustment of the transfer day. As described by Richard Scott in another review of this series, fully expanded day 5 or day 6 blastocysts have similar implantation and PRs during FET cycles, whereas the PR with day 6 blastocysts in fresh cycles is reduced. Similarly, endometrial biopsy and assessment of the endometrial development stage by several presently available techniques facilitates personalized ET depending on the presumptive timing of the window of implantation and the stage of development of the embryo.

When performing FET, it is usual to administer E until the endometrial thickness on ultrasound has reached approximately 0.8 cm and then to add P for the number of days proportional to the stage of development of the embryo being transferred (8). It is the presumption that after E priming, exposure to P for a specific number of days will result in an endometrial lining that is appropriate to support implantation of a cleavage stage embryo or blastocyst. However, this assumption may not always be correct. An endometrial biopsy that shows a difference of more than 2 days between the histologic dating and actual day after ovulation is considered to be "out of phase" (9). In previous publications, out of phase endometrium was found in 5%–50% of patients (10–12). These studies were performed during natural cycles, and the large variation in results may have been related to subjective historic or other means of determination of the day of ovulation (urinary LH surge test kits) that might not be completely accurate. Therefore the out of phase label might have been the result of inaccurate determination of the time of ovulation. In addition, it is possible that there is variability from cycle to cycle even in fertile women in luteal phase endometrial development (13). Murray et al. (13) found that up to 26% of endometrial biopsies 6–10 days after ovulation were 2 or more days delayed and based on these observations decided that the Noyes criteria were not accurate or reliable. However, as described later, it may be the window of implantation that is not always reliable rather than the histologic dating.

Any doubt of when the luteal phase actually starts can be obviated by hormonal endometrial preparation for FET. In this case, most patients receive high dose E treatment administered during the follicular phase that inhibits gonadotropin secretion and prevents follicular development and ovulation.

Alternatively, a GnRH agonist is administered to suppress gonadotropin secretion during endometrial preparation. Consequently, the start of the luteal phase can be determined exactly, as it occurs when P is added to the E replacement. Using E and P prepared cycles, an endometrial biopsy on the sixth day of P administration should be histologically determined to be about day 20 of an idealized 28-day cycle. Using microarray molecular analysis (endometrial receptivity assay), Simon et al. found that about 25% of the endometrial biopsies were delayed in relation to day 20 (14). Similarly, using simple endometrial dating of endometrial biopsies ("Noyes criteria"), we showed exactly the same result (i.e., about 25% of samples were delayed) (15). Both of these results concur with the findings of Murray et al. (13) suggesting that the criteria of Noyes are accurate but there is delayed endometrial development in the luteal phase in about a quarter of women. Based on these findings, we believe that it is timely to consider a large randomized controlled trial (RCT) to determine whether a mock cycle with endometrial biopsy and endometrial receptivity assay plus or minus endometrial dating may be useful in the first FET cycle to improve PRs compared with nonbiopsied cycles. Such a study, if positive, would support the concept of personalized FET by adding 1–3 days of P and delaying FET in women with demonstrated delayed endometrial development. Potentially confounding variables in all cases of FET are the route of administration and dose of the E and the P, as reviewed later. Much more research into the methodology of endometrial preparation is required before we will have a clear picture of how to provide consistent and appropriate endometrial preparation.

Another consideration, even if timing of the window of implantation is correct, is uterine activity at the time of ET, either spontaneous or resulting from traumatic or difficult ET. Multiple subendometrial contractions manifested as endometrial waves in the luteal phase are associated with a lower PR as first demonstrated by Fanchin and colleagues (16) in France. Subendometrial contractions might also explain some ectopic pregnancies (EPs) that occur with ET. Embryos are placed in the midendometrial cavity under ultrasound guidance. Therefore, the only way to explain the occurrence of a tubal EP is the occurrence of endometrial activity that pushes the embryo up into the fallopian tube. This hypothesis is supported by sonographic studies that determined the movement of a suspension of galactose microparticles placed in the endometrial cavity under ultrasound guidance. This study demonstrated the movement of the microparticles into the cervix or into the fallopian tubes in certain patients, consistent with abnormal uterine contractility (17).

It is known that E increases uterine contractility and subendometrial wave activity and that P antagonizes this action to quiet the uterus and reduce endometrial waves. In controlled ovarian stimulation for assisted reproductive

technology (ART), supraphysiologic levels of E are associated with more frequent endometrial waves than seen in natural cycles (18). The effect of P to quiet the uterine wave activity may be duration and dose related and may be one of the reasons for better PRs with day 5 compared to day 3 ETs. In addition, in cases of potential P resistance (e.g., endometriosis), there is a possibility of increased endometrial wave activity and reduced implantation rates (19). Based on this hypothesis and the study of Fanchin et al. (16), we now routinely count the number of endometrial waves per minute on the day before thawing embryos for FET. We do this wave count in all women undergoing FET cycles, whether on vaginal or IM P. If the number of waves is two or fewer per minute, the embryo warming and transfer is planned for the next day. Alternatively, if the wave count is more than two, we administer an extra dose of P as an IM injection of P in oil in the evening and recheck the wave count in the morning. We thaw the embryo for transfer if the morning wave count is less than three per minute. This suggested management is empirical and is based on the assumption that augmenting P exposure when the contraction frequency is high should result in improved results. However, a randomized and blinded study should be done to confirm that hypothesis.

In conclusion, the window of implantation in hormonally prepared cycles for FET has been thought to be relatively consistent but recent data from both transcriptomic microarray as well as from simple histologic endometrial dating have shown that the receptive phase may be delayed in about one of four women. In that case, delaying FET according to the endometrial delay (personalized ET) may lead to improved PRs, especially in women with multiple failed cycles and apparently good quality embryos. Further research will be necessary to determine whether a personalized evaluation of endometrial wave activity, with augmentation of P administration as indicated, could also improve PRs in a proportion of women having excessive wave activity.

P SUPPLEMENTATION IN THE NATURAL, MODIFIED NATURAL, AND PROGRAMMED FET CYCLES: IM VERSUS VAGINAL VERSUS NONE

With significant improvements in cryopreservation technology (vitrification) the number of FET IVF cycles is increasing and may soon surpass in numbers as well as success rates those of fresh stimulated IVF cycles. Increasing numbers of elective single ETs are also resulting in greater numbers of frozen embryos (blastocysts) available for subsequent FET cycles. The optimal endometrial preparation in FET cycles thus assumes utmost importance for insuring the best FET outcomes.

Reliable data are essential for defining the optimal endometrial preparation protocols for FET cycles. Most reliable data comes from randomized studies (RCT) with adequate power to ascertain differences in outcomes, whereas retrospective studies, even with large numbers, are notoriously unreliable because of unaccounted biases and confounders. A good example of retrospective studies arriving at diametrically opposite conclusions with respect to vaginal versus IM P supplementation for stimulated IVF cycles are those of Papaleo et al. (20) and Ho et al. (21). The former showed lower PRs with vaginal P administration, whereas the latter study showed higher PRs with vaginal P supplementation. The truth, however, is that both preparations are equal in efficacy for stimulated IVF cycles, with vaginal preparations being preferred by patients, as was demonstrated by three randomized and adequately powered studies (22–24) from three different countries. It is with the acknowledgment of the paucity of adequately powered randomized data and heterogeneity of the retrospective reports that we approach the question of the optimal P support in FET cycles.

NATURAL VERSUS MODIFIED NATURAL VERSUS PROGRAMMED (ARTIFICIAL) FET REGIMENS

Natural cycle FET involves frequent monitoring of urine and/or blood LH levels, early luteal serum P levels, and ultrasound monitoring of the developing dominant follicle. For optimal FET results precise identification of the LH surge and adequate natural corpus luteum (CL) function are required. Precise identification of the LH surge is difficult for logistical reasons and adequate natural CL function can be assumed only with perfect ovulatory cycles.

Modified natural cycles involve ultrasound monitoring of the developing follicle, measurements of the endometrial stripe, and monitoring of serum hormone levels followed by hCG administration when the lead follicle is ≥ 17 mm and the P level is low. This approach allows for precise definition of the ovulation trigger for scheduling FET, and also provides luteal support in the form of hCG in case of possible luteal dysfunction.

It is important to make a distinction between natural and modified natural FET protocols because of the inherent additional luteal phase support provided by hCG injection in the modified natural cycle protocols. Unfortunately, most reports in the literature with the "natural FET" designation in the title actually describe the monitored natural FET cycles with hCG triggers. We will adhere to the exact definitions for natural versus modified natural FET cycles in the later discussion.

Programmed FET regimens use suppression of natural menstrual cycle with or without the use of GnRH agonist and require exogenous E and P replacement to achieve adequate proliferative and secretory changes in the endometrium in preparation for implantation and early pregnancy support. Various E and P preparations (oral, vaginal, transdermal, and IM) have been used successfully in programmed FET cycles.

P SUPPLEMENTATION/REPLACEMENT IN FET CYCLES

A large RCT (N = 435) comparing PRs in natural FET cycles with vaginal P supplementation (400 mg twice/day starting on the evening of ET–day 3 embryos) versus no P supplementation observed a significantly greater live birth rate in supplemented cycles (25). A smaller RCT (N = 102) compared natural FET (day 3 freeze) with IM P supplementation versus

no supplementation and found no statistical differences in clinical PRs, although there was a 6% lower clinical PR in the nonsupplemented group, which did not reach statistical significance, possibly due to the small sample size (26).

A recent pilot RCT (N = 159) compared natural FET cycles without luteal support to programmed FET cycles with GnRH agonist down-regulation followed by oral E and vaginal P (pessaries) and found no statistical differences in implantation rates and live birth rates between the two group. However, six patients initially randomized to the natural FET group had to be excluded from the study because of failure to detect a spontaneous LH surge (27).

A larger but retrospective study (N = 417) comparing pregnancy outcomes in natural versus programmed FET cycles using GnRH agonist down-regulation and oral E and vaginal P preparations (day 3 freeze) reported similar pregnancy outcomes, but did not include cancellation rates due to the inability to precisely detect an LH surge. This illustrates the limitations of the natural cycle FET for oligoovulatory patients as well as for logistical reasons (28).

Meta-analyses and reviews of retrospective reports comparing modified natural FET cycles with or without luteal P supplementation in any form (vaginal or IM) did not find significant differences in FET outcomes. Furthermore, no significant differences with respect to pregnancy outcomes were found between modified natural FET preparations and programmed FET cycles with or without the use of GnRH agonists (29–32). This suggests that hCG administration in modified natural FET cycles provides luteal support that is comparable to either IM or vaginal P preparations in programmed cycles, and therefore P supplementation in a modified natural FET cycle may not be needed at all.

Programmed FET regimens that use suppression of the natural menstrual cycle with E and P replacement (with or without the use of GnRH agonists) allow for the most scheduling flexibility and often the least amount of monitoring. In the absence of adequately powered RCTs there has been much controversy regarding the use of vaginal versus IM P supplementation in programmed FET cycles. Two small prospective studies (33, 34) from 1999 and 2000 comparing the use of IM P and P vaginal gel in donor egg recipient cycles showed similar results.

A recent small RCT (N = 76) using a programmed down-regulation FET approach showed similar life birth rates between patients randomized to IM P and those randomized to a sequence of oral micronized P before ET and vaginal suppositories (200 mg 3 times/day) after transfer. To strengthen their conclusions Leonard et al. (35) provided similar results of a larger retrospective analysis (N = 508) from their program and included them in the final publication.

A larger body of data on IM versus vaginal P supplementation for programmed FET cycles is retrospective and conflicting. Three retrospective analyses reported higher live birth rates in the IM P supplemented programmed FET (autologous and donor egg) cycles (36–38), whereas four reports (39–42) showed no difference in live birth outcomes.

Most of these retrospective reports have significantly unequal numbers in the IM P group versus vaginal P groups; they are also heterogeneous with respect to the day 3 versus day 5 transfers as well as to specific vaginal P preparations, doses, and timing of administration. The two largest of these retrospective studies—Kaser et al. (38) and Shapiro et al. (42)—reported results with day 3 FET and day 5 FET, respectively. Kaser et al. (38) found higher live birth rates with IM P for day 3 FET, whereas Shapiro et al. (42) showed equal PRs with vaginal compared with IM P preparations for day 5 FET.

It has been well documented in elegant pharmacokinetic studies that absorption into the endometrium is superior with the vaginal compared with IM P administration, whereas higher serum P levels are measured after the IM injections (43–45).

Traditionally higher serum P levels were presumed to be better for the FET outcomes. However, two recent retrospective studies showed that increasing IM P doses to achieve higher serum levels does not translate into improved outcomes (46), and that high P levels (>20 ng/mL) on the day of transfer of single euploid blastocysts were associated with lower ongoing PRs and lower live birth rates (47).

In the absence of data on clear superiority of either vaginal or IM P preparations in programmed FET cycles patients' acceptance, convenience, potential complications, and costs should factor into the determination of the optimal FET protocol. Vaginal preparations have been demonstrated to be better tolerated than IM P (24), but new SC P preparations and oral P preparations currently available in Europe and Asia also hold promise.

Two RCTs (48, 49) have already demonstrated noninferiority of the SC P preparation compared with the vaginal preparations in stimulated cycles, and two other RCTs (50, 51) demonstrated equal efficacy of oral dydrogesterone and vaginal P in stimulated cycles with patients preferring the oral route of administration. Two retrospective reports (52, 53) from China suggested equal efficacy of oral dydrogesterone with vaginal and IM P preparation in FET cycles.

A properly powered RCT is much needed to establish the best endometrial preparation protocol for FET cycles. Until then, based on the limited prospective data and conflicting retrospective results we have to conclude the following:

1. Natural FET cycles benefit from vaginal P supplementation starting after ET. They are most appropriate for patients with regular ovulatory cycles who are able to comply with strict regimen of frequent urine and blood hormonal measurements.
2. Modified natural FET cycles require ultrasound monitoring and blood hormonal measurements for optimal timing of hCG trigger. Luteal phase support with P does not appear to be necessary because of the luteotrophic effect of hCG. P support after ET could be optional and should use the most convenient and cost effective P preparation.
3. Programmed FET cycles are the most convenient with respect to limited monitoring requirements and ease and flexibility of scheduling. However, they have not been shown to be superior to properly timed natural or modified natural FET protocols. The optimal form of P supplementation has not been established from available data. Patients' preference and convenience, as well as costs should be

considered when choosing either vaginal or IM P preparations.

4. Alternative options for P supplementation in FET cycles—SC and oral—should be evaluated with adequately powered randomized trials.

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