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