The endometrium in assisted reproductive technology: How thin is thin?

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ABSTRACT

A thin endometrium is encountered infrequently (2.4%) in assisted reproductive technology cycles. When it does occur it is a cause of concern as it is associated with lower implantation rate and pregnancy rate. Though pregnancies have been reported at 4 and 5 mm it is apparent that an endometrial thickness <6 mm is associated with a trend toward lower probability of pregnancy. Hormone replacement therapy – frozen embryo transfer (FET) cycles appear to give better results due to an improvement in endometrial receptivity (ER). The etiology of thin endometrium plays a significant part in its receptivity. A number of treatments have been tried to improve endometrial growth, but none has been validated so far. Confirming ER of a thin endometrium by an ER array test before FET offers reassurance.

KEY WORDS: Assisted reproductive technology, endometrial receptivity array, frozen embryo transfer, thin endometrium

INTRODUCTION

The ability to predict pregnancy outcome following *in vitro* fertilization (IVF) remains elusive and has led to a search for predictive markers. Maternal age, ovarian reserve measurement, and markers of endometrial receptivity (ER) have been evaluated in this context. ER is integral to implantation so identification of an accurate marker of implantation would be highly beneficial in assisted reproductive technology (ART). Despite enormous research in the field of human embryo implantation, the ideal marker of ER remains indefinable. Lack of accuracy, predictive value, and invasive nature of the biochemical and histological markers of ER limit their clinical applicability. Sonography by virtue of its noninvasive nature and universal availability is the modality most often used for assessment of ER in ART.

Endometrial changes through the menstrual cycle reflect the steroid status of the cycle. The functional layer of the endometrium starts growing under the influence of estrogen (E) till it reaches a maximum at the onset of the luteinizing hormone (LH) surge. The pre- and post-ovulatory rise of progesterone (P4) herald's secretory changes in the endometrium and these are paralleled by changes in the endometrial pattern (EnP). On ultrasound endometrial thickness increases in the follicular phase and endometrial character changes from a hypoechoic trilaminar one to a compact hyperechoic look postovulation. A trilaminar endometrium on the day of ovulation trigger is associated with an increased probability of pregnancy while a hyperechoic character signals failure.[1]

The parameters used to evaluate ER with a traditional two-dimensional ultrasound are an assessment of endometrial thickness (Eth) and EnP. With the advent of three-dimensional and four-dimensional ultrasound, additional factors have been studied to improve the predictive value of this investigative modality. These include

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measurement of endometrial volume and Doppler sonography of uterine and sub-endometrial blood flow.[2,3] Interestingly, endometrial thickness and pattern still remain the most researched parameters for their predictive value in IVF.

Endometrial thickness (Eth) is measured by transvaginal ultrasound as the maximal distance between the echogenic interfaces of the myometrium and endometrium in the plane through the central longitudinal axis of the uterine body. An endometrial thickness of <7 mm at the time of embryo implantation is considered suboptimal in ART. With respect to EnP and ER[4] a triple line appearance or multilayered endometrium on the day of the ovulation trigger is defined as Grade A or receptive while a homogenous appearance or nonmultilayered endometrium is defined as Grade C or nonreceptive. Progesterone secretion initiates changes in the endometrium that are reflected as a homogenous character near the junctional zone and a well-defined central echogenic line. This EnP seen on the day of ovulation trigger is defined as Grade B.

WHAT DO WE UNDERSTAND BY THE TERM "THIN ENDOMETRIUM" AND WHAT IS ITS RELEVANCE IN ASSISTED REPRODUCTIVE TECHNOLOGY?

A thin endometrium is mostly defined as an endometrial thickness of <7 mm on ultrasound[5-10] although a cut-off value of 6 mm[11-13] and 8 mm has also been used.[14]

Though controversial endometrial thickness has been used to predict the possibility of pregnancy in ART cycles; a thin endometrium being associated with poor success rates after IVF irrespective of the causative factor. However, pregnancies have also been reported at an endometrial thickness of 4 mm and 5 mm[15-17] suggesting that receptivity may not necessarily be related to Eth. A thin endometrium is seen more often in older women probably because of decreased vascularity. An incidence of 5% has been reported in women <40 years and 25% beyond age forty in natural cycles.[18] Kasius *et al.* 2014[19] reported an incidence of 2.4% in their meta-analysis that included 1170 patients undergoing IVF.

Kumbak *et al*., 2009,[6] looked at the cycle characteristics and outcomes of 175 patients with an endometrial thickness of <7 mm on the day of oocyte retrieval. Patients were stratified according to three endometrial thickness groups ≥4 mm and <5 mm, ≥5 mm and <6 mm, and ≥6 mm and <7 mm. Pregnancy rate (PR) and implantation rate (IR) did not show a statistically significant difference among the three groups though an increasing trend in values was observed as endometrial thickness increased. The clinical PR (CPRs) and miscarriage rates (MRs) of the study group were compared with 5573 patients undergoing IVF during the same period who had an endometrial thickness of ≥7 mm. The CPR's were 26% and 51% ($P < 0.0001$), MR 31% and 17% ($P = 0.02$) in patients with endometrium <7 mm and >7 mm, respectively. The CPR, IR, and live birth rate (LVBR), per embryo transfer (ET) of patients with a thin endometrium, were further assessed according to age, number of oocytes retrieved and embryos transferred. Significantly better results were obtained when the patient's age was <35 years or the number of retrieved oocytes was >5 or the number of embryos transferred was three or more. The authors suggest that if the endometrium is <7 mm on the day of oocyte pick-up, the patient should be offered total embryo freezing unless the number of oocytes recovered is >5, the number of embryos available for transfer ≥3. Even though this study supports the view that an endometrium <7 mm compromises chances of pregnancy, results based on stratification by age, oocyte numbers, and number of embryos transferred indicate that endometrial thickness is not the only determinant of treatment outcome.

A contrary view was given by Kasius *et al*., 2014,[19] based on a systemic review and meta-analysis of 1170 studies (22 of which were of moderate quality). Their review suggests that Eth cannot be used as a parameter to decide on cycle cancelation, freezing of all embryos or discontinuing IVF treatment. A thin endometrium (≤7 mm) occurred infrequently - 2.4% of the reported cases (260/10 724) and the estimated summary receiver operating characteristic curve indicated a virtually absent discriminatory capacity of Eth in the prediction of pregnancy. However, there was a trend toward lower ongoing pregnancy and LVBRs for these women (odds ratio [OR] 0.38 [95% confidence interval [CI] 0.09–1.5]). The probability of clinical pregnancy for Eth ≤ 7 mm was significantly lower compared with that for an Eth > 7 mm (23.3% vs. 48.1%, OR 0.42 [95% CI 0.27–0.67]). The positive predictive value for the outcome of clinical pregnancy was 77%, and the negative predictive value was 48%. One can conclude from this review that Eth can give us probability but cannot be predictive of pregnancy.

The adverse effect of controlled ovarian hyperstimulation (COH) on ER is well established, and elective freezing of embryos with the subsequent transfer in a hormone replacement therapy (HRT) cycle is advocated to improve implantation. The effect of endometrial thickness on PR in a frozen embryo transfer (FET) cycles has also been examined. El-Toukhy *et al*., 2008,[5] found that an endometrial thickness of 9–14 mm measured on the day of *P* supplementation was associated with higher implantation and PRs compared with an endometrial thickness of 7–8 mm. Dix and Check, 2010[20] in a retrospective

analysis looked at PR in patients with Eth of <6 mm. Of the 35 patients, there were only three pregnancies and only two patients delivered. The overall PR was 8.5% per transfer and LVBR 5.7% per transfer PR in FET group was higher being 14.2%. The maximal thickness at which a patient achieved LVB was 5 mm in the FET group. A recent study by the same group[21] compared the LVBR, PR, and IR between fresh and FET in endometrium <6 mm. The IR and LVBR at 4–5 mm in fresh versus frozen transfer cycles was 10.6% versus 27.2% ($P = 0.079$) (IR) and 10% versus 36% ($P = 0.0325$) (LVBR) being higher in the HRT-FET cycles. ET was not attempted when endometrium was <4 mm. Both the studies from this group imply that an HRT replacement cycle could mitigate the negative effect of COH even in patients with a thin endometrium.

Oocyte donation cycles are ideal to measure the independent effect of Eth as a parameter of ER because there is lower variability of embryo quality. Dain *et al*., 2013,[22] studied the effect of Eth on reproductive outcome in oocyte donation cycle using 6 mm and 8.2 mm as the cut-off for a thin endometrium. There were no statistically significant differences in CPR (29.6% vs. 30.0%) and LVBR (16.7% vs. 23.6%) in women with endometrium <6 mm compared with Eth >6 mm, respectively. However, more live births were observed for an Eth cut-off of 8.2 mm than for thinner endometrium. It is possible that a thin endometrium is unable to support pregnancy development after implantation, resulting in more miscarriages due to intrauterine (IU) fetal deaths. It was also observed that a lower proportion of patients with endometrium thinner than 6 mm exhibited EnP Grade A and a higher proportion exhibited Grade C.

Embryo quality plays a major role in implantation and is one of the determining factors for pregnancy outcome. Gingold *et al*., 2015,[14] sought to evaluate the relationship of endometrial thickness (EnT) and EnP to pregnancy outcome after euploid ET. Having transferred only euploid embryos after preimplantation genetic screening they found that Eth (≤8 vs. >8 mm), on the day of trigger or ET, had no significant correlation with IR or clinical outcomes across all age groups (23.4–44.4 years, mean: 36.1 ± 4.0 years) in either fresh or FETs. However, a completely homogenous EnP at trigger did correlate with a low IR. The Eth ranged from 4.4 to 17.9 mm (mean: 9.7 ± 2.2 mm) at fresh ET day, and from 4.2 to 17.7 mm (mean: 9.1 ± 2.1 mm) at FET day. The subset of patients with endometrium ≤7 mm was too small to analyze for statistical significance. The authors also suggest that these results may not apply to patients whose Eth or EnP is altered because of endometrial pathology (e.g., from Asherman's syndrome, IU tuberculosis, or an autoimmune disorder). Endometrial damage by disease can lead to reduced vascularity and fibrosis.

Let us examine the reasons for thin endometrium, the modalities used to attempt correction/improvement and the pregnancy outcome if ET is attempted after defining the transcriptomics of the window of implantation (WOI) in patients with Eth of 6 mm or less.

CAUSES OF THIN ENDOMETRIUM

Thin endometrium can result from various factors the most common being inflammatory and iatrogenic. Poor vascularity and low estradiol values can also lead to poor endometrial growth. The endometrium can also be inherently thin in some women.[22]

- Inflammatory causes: Acute or chronic infection can lead to the destruction of the basal layer of the endometrium. In India, genital Koch's is the most common cause of thin endometrium. Since healing takes place by fibrosis, it leads to the destruction of the endometrium and shrinkage of the uterine cavity. Regeneration of endometrium even after complete treatment is very difficult as fibrosis destroys the basal layer
- Iatrogenic: Surgical – repeated or vigorous curettage damages the basal layer of endometrium. Hysteroscopic myomectomy, polypectomy, or laparoscopic myomectomy where the cavity is opened may lead to IU adhesions. Medical – indiscriminate use of drugs such as clomiphene citrate
- Idiopathic: Thin endometrium may not necessarily be secondary to a disease process. It can result from individual uterine architecture[23] or the intrinsic properties of endometrium that affect its growth.[24]

Miwa *et al*., 2009,[25] demonstrated that thin endometrial were characterized by poor growth of glandular epithelium, high uterine blood flow impedance, decreased vascular endothelial growth factor (VEGF) expression, and poor vascular development. They postulated that a high blood flow impedance of radial arteries acting as the trigger impaired the growth of the glandular epithelium and resulted in a decrease in VEGF levels in the endometrium. Low VEGF, in turn, causes poor vascular development, which further decreases blood flow in the endometrium. This vicious cycle leads to a "thin" endometrium.

MODALITIES TO IMPROVE REFRACTORY ENDOMETRIUM

Numerous treatments have been tried to improve refractory endometrium, but success has been limited. Currently, evidence-based medicine has not validated any specific treatment. The most popular ongoing treatments will be discussed.

Intra-uterine granulocyte colony-stimulating factor

The human endometrium expresses granulocyte colony-stimulating factor (G-CSF) mRNA and its receptor throughout the menstrual cycle. G-CSF may, therefore, play a physiological role in endometrial development through interactions with other cytokines and ovarian steroid hormones. Estrogen may be necessary to provide nutrition to the endometrium after stimulation by G-CSF.[26] It has been demonstrated that G-CSF can increase mesenchymal and hematopoietic stem cells in the bone marrow.[27,28] The rationale for IU G-CSF instillation to improve endometrial growth stems from the understanding that the human endometrium contains a small population of mesenchymal stem-like cells that could be responsible for endometrial cyclical growth and reconstruction.[29] Diminished endometrial stem cell (ESC) numbers or function may compromise endometrial growth. It is possible that subsequent to injury some cells involved in endometrial growth may become quiescent while others maintain basal growth resulting in thin endometrium. It is proposed that G-CSF may stimulate ESCs or mobilize bone marrow stem cells promoting endometrial development.[30,31] Gleicher *et al*., 2011,[32] was the first to report that IU G-CSF instillation improves Eth. Subsequently, many studies have been published some reporting improvement[26,33,34] while others do not show any difference.[35,36] The dose and time of instillation of the drug are yet to be standardized. A dose of 300 μg G-CSF or 100 μg/0.6 mL recombinant G-CSF is administered in the proliferative phase, on the day of human chorionic gonadotropin (HCG) administration, on the day of ovulation or day of administration of progesterone.

Even if we accept that G-CSF improves Eth can one or two instillations improve PR? Studies reporting an improved Eth with G-CSF were unable to show a significant improvement in PR.[33,34,37] The only study that reported a significant increase in IR and PR performed FET and had younger patients in their study.[26]

G-CSF instillation is of limited value in patients with IU adhesions where damage to the endometrium is extensive. Perhaps repeated IU G-CSF perfusion or administration in combination with other cytokines is feasible, but further studies are necessary to determine the perfect dose and treatment duration.

Extended estrogen support

Endometrial thickness can be improved by extending the estrogen administration for 14–82 days, in HRT-FET cycles.[38]

Human chorionic gonadotropin priming in the follicular phase

150iu HCG given daily for 7 days starting from day 8 to 9 of estrogen therapy has been suggested by Papanikolaou *et al*.[39] to improve Eth. Their rationale was that HCG administration might have a positive effect on the endometrial HCG/LH receptors. Their study suggests that apart from improvement in Eth of approximately 20% there may be an improvement in ER.

Drugs that increase endometrial blood flow have been administered individually or in combination to improve Eth. Pentoxyfilline 800 mg/day and tocopherol 1000 mg/day given over several months,[40] sildenafil 100 mg/day given as vaginal passary, l-arginine 6 g/day,[41] and low dose aspirin 75 mg/day. None of these therapies have met with much success.

MISCELLANEOUS TREATMENTS

Occasional reports of IU autologous platelet rich plasma infusion, IU administration of bone marrow stem/progenitor cells, luteal phase support with GnRH agonist, pelvic floor Neuromuscular electrical stimulation for improving Eth are found in literature, but none of the treatments have been substantiated.

Regenerative medicine – numerous research units are working on the use of stem cell therapy for regeneration of the endometrium. So far, it remains a research protocol and has not been cleared for routine clinical use.

IS ASSESSMENT OF FUNCTIONALITY IMPORTANT?

As suggested by various case reports and studies thin endometrium is not necessarily nonreceptive. The ER array (ERA) test is a molecular test that defines the window of ER.[42] This test can be used to confirm the receptivity of a thin endometrium before planning FET. In case of a changed WOI, a personalized ET can be performed. A study done on patients with thin endometrium ≤6 mm patients revealed that ERA was nonreceptive in 23% (3/13) and receptive in 77% (10/13). The proportion of receptive to nonreceptive endometrium was not different from patients with Eth >6 mm. The overall PR in the thin endometrium group was who underwent ET after ERA was 33.3% (6/9).[43]

How should we interpret these results for use in our clinical practice?

Despite conflicting reports, it is clear from these studies that EnP on the day of ovulation trigger is a better indicator of the probability of pregnancy than endometrial thickness. Whether this applies to HRT-FET cycles as well has not been well substantiated. Fortunately, a thin endometrium is encountered infrequently in ART cycles. When it does occur, it is a cause of concern as it is associated with lower IR and PR. An endometrial thickness of 7 mm seems to be

the cut-off value defined by most authors. Some studies have shown that implantation is not compromised when the endometrium is ≥6 mm with a trilaminar pattern. Though pregnancies have been reported at 4 and 5 mm Eth, it is apparent that a thin endometrium as defined by an Eth of <6 mm is associated with a trend towards the lower probability of pregnancy. HRT-FET cycles appear to give better results due to improved ER. It is also important to understand that the etiology of the thin endometrium may play a significant part in its receptivity. A thin endometrium subsequent to endometrial destruction by an inflammatory process may be less receptive than one that is due to the individual architecture of the uterus.

The burning question regarding treatment of thin refractory endometrium remains unanswered. A number of treatments have been tried but none validated so far. IU insertion G-CSF is currently the most popular treatment, but results are not consistent between various studies. Sildenafil, tocopherol, aspirin, and L-arginine have been used to improve endometrial vascularity. Extended estrogen therapy has also been attempted to improve Eth. Use of GnRH analog before HRT is not recommended as it decreases endometrial vascularity resulting in poor endometrial growth. Among these treatments, the one that we have personally found effective is the use of repeated HRT cycles before FET to stimulate regeneration of the endometrium. Endometrial regeneration is seen to occur more frequently in women who have had a prior pregnancy. Thin endometrium resulting from an inflammatory process may improve a little but generally remain unresponsive. The use of ERA to define the window of ER before ET is helpful. Women with thin endometrium may be at risk of abnormal placentation after achieving pregnancy. The possibility of early miscarriage, placenta accreta, and postpartum hemorrhage should be discussed.[38] The future answers may lie in regenerative medicine.

CONCLUSION

Though endometrial thickness is not predictive of pregnancy after IVF the probability of pregnancy is reduced with an endometrial thickness below 6 mm. The reasons for low implantation could be a high impedance blood flow of the radial arteries leading to poor endometrial glandular growth and poor angiogenesis subsequent to decreased VEGF secretion. In addition to lower implantation, the process of invasion may be hindered due to the lack of an adequate endometrial bed. This in turn increases chances of poor placentation and appropriate vascularization leading to early abortions even if pregnancy is established. Placenta accrete has also been reported Many modalities have been applied to improve endometrial thickness but their effectiveness remains controversial. Transfer of embryos to an endometrium prepared by HRT seems to yield better results than fresh ET. ERA may be applied in such patients to ensure that the embryo is transferred to a receptive endometrium.

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