

Current Strategies to Manage a Thin Endometrium

This article was published in the following Scient Open Access Journal:

Women's Health & Gynecology

Received April 10, 2016; Accepted April 18, 2016; Published April 22, 2016;

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Introduction

One of the key factors for successful embryo implantation is the presence of a receptive endometrium. The achievement of a receptive endometrium involves proliferation and thickening of the endometrium, synchronization of the micro architectural changes of the endometrium with the stage of embryo development and the presence of a proper cytokine environment [1]. Despite its complexity, the process of achieving endometrial receptivity can be attained in most women by a successive exposure of the endometrium to estrogen and progesterone and is likely similar in spontaneous pregnancy as well as with fertility treatment [2].

In recent years, significant advancement has been made in the understanding of embryo development and assessment as well as in the technique of embryo culture and cryopreservation. The same cannot be said about our understanding of endometrial development, implantation and the techniques to improve endometrial quality and receptivity.

The endometrium is a dynamic tissue that responds to changing hormonal signals throughout the cycle. The changes in the endometrial composition are expressed in alteration in gene expression, micro architectural morphological changes as well as in protein and hormone secretion. These factors combine together to construct the "window of implantation" a short period of time during the luteal phase in which the endometrium is receptive [3]. Endometrial thickness is an expression of the proliferative capacity of endometrium in response to unopposed estrogen during the follicular phase of the cycle. Normal development of the endometrium along the reproductive cycle can be characterized by the successive morphological changes occurring during the cycle. Noyes [4] describes in his work the histologic changes during the menstrual cycle. The criteria established by Noyes are still considered the gold standard for endometrial dating and assessment of synchronicity. In the proliferative phase the functional layer of the endometrium proliferates under the effects of estrogen while post ovulation the increased progesterone secretion induces the secretory transformation of the endometrium, expressed by endometrial edema and decidualization. This series of carefully orchestrated changes are critical for endometrial receptivity and implantation.

Immediately after ovulation the spiral arteries of the functional endometrium constrict and cause a reduction in blood flow to the functional layer [5]. The reduced blood flow decreases oxygen tension in implantation surface of the endometrium; this low oxygen environment appears to be more welcoming to the embryo. This assumption is strengthened by the finding that embryos cultured in vitro under low oxygen concentration show superior quality and outcome. This phenomenon is attributed to lower levels of free oxygen radicals and is now the standard practice in IVF laboratories.

It is still unclear why a thin endometrial lining reduces the chance of a successful implantation. A recent editorial written by Robert Casper [6] provided a novel explanation to the poor outcome associated with a thin endometrium. According to this theory when an embryo is placed over a thin endometrium it is closer the more vascularized stroma and therefore exposed to a much higher oxygen tension. An alternative explanation suggests that the cause of a permanently thin endometrium is a dysfunction in the estrogen receptor that is involved both with endometrial proliferation and embryo implantation thereby impairing both endometrial proliferation and implantation [6].

Several methods were suggested to try and predict the optimal endometrial

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conditions for implantation in assisted reproductive techniques (ART). Endometrial thickness is the most widely accepted method to assess endometrial receptivity. However, due to its poor sensitivity and predictive value, the search for better methods for endometrial assessment is ongoing. Currently most of the newly developed tests are still controversial and lack certainty regarding their predictive value and reproducibility. These methods include endometrial histologic dating [3,7], sonographic assessment of endometrial volume [8] uterine and sub-endometrial blood flow [9,10] endometrial receptivity array (ERA) [11,12], Sub-endometrial contractility waves, matrix and other methods of endometrial assessment that are currently being investigated.

Trans-vaginal sonographic measurement of endometrial thickness is a non-invasive technique that enables the clinician to follow endometrial development. It is a simple and widely accepted method to estimate endometrial receptivity. The test is well-established and is now considered standard of care. In IVF cycles, most physicians would prefer not to transfer an embryo to a patient with thin endometrium, often leading to cancellation of fresh as well as frozen embryo transfers.

Generally, thin endometrium is associated with a low pregnancy rate. The widely accepted cut-off to define a thin endometrium is 7 mm in the pre-ovulatory phase [13-15]. Other cut-offs reported in published studies usually range between 5-8 mm [16-18]. Several studies reported on a higher pregnancy rate achieved with a blastocyst stage embryo transfer when endometrial thickness was >9 mm [19]. Similar endometrial thickness was found to be the cut-off to predict pregnancy following analysis of 1933 IVF cycles [20]. Dain, et al. reported on a higher live birth rate with endometrium 9.1-10 mm in donor egg recipients [21]. Al-Ghamdi, et al. [22] found a positive correlation between endometrial thickness and pregnancy rate. However pregnancies were reported despite having endometrial thickness as thin as 4 mm [23]. In a Meta-analysis conducted by Kasius, et al. endometrial thickness by itself was not found to be a positive predictive value for pregnancy incidence [24] even with different cut-off standards tested.

Sonographic assessment of endometrial pattern is another parameter used to define endometrial receptivity. A recently published retrospective analysis of 356 embryo transfer cycles demonstrated no correlation between implantation and pregnancy rates and endometrial thickness; measured on the day of the trigger injection as well as the day of transfer of euploid embryos. In the same study endometrial pattern was evaluated, showing unfavorable results with mid-secretory pattern at day of triggering [25]. Earlier reports related follicular (tri-laminar) endometrium with higher pregnancy rate when endometrial thickness measurement was 7-14 mm [20,26]. However, endometrial pattern evaluation continues to provide inconsistent results whether it is investigated as a sole factor or in combination with endometrial thickness.

The incidence of thin endometrium varies according to the chosen cut-off. Bu, et al. [27] found the incidence of thin endometrium <7 to be 1.49-2.5% in high responders and poor responders respectively. An association between age and endometrial thickness has been reported by Sher, et al. According to their study, thin endometrium was more prevalent among

older women; it has been reported in 5 % of women <40 years of age and in 25 % of 41-45 years old women [28].

A certain percentage of cases of persistently thin endometrium may be explained by previous iatrogenic intervention. Intra-uterine procedures that damage the endometrium or its blood supply may lead to adhesion formation and impaired development of the endometrial layers. Dilatation and curettage is notorious for its associated risk of permanently thin endometrium and intra-cavitary adhesions, especially if done post-partum [29]. Abnormal response to estrogen as a cause of a persistently thin endometrium is well documented, and might be a result of several different mechanisms; the anti-estrogenic effect of clomiphene citrate [30], prolonged exposure to combined birth control pills [31] and progesterone contraception. The continuous exposure to the progestin in the oral contraceptives leads to down regulation of estrogen receptors and as a result induces atrophy of the glandular epithelium and form a distinct morphological pattern of atrophic glandular epithelium against a background of secretory glands [32].

Regardless of the reason, persistently thin endometrium is a challenging entity for the clinician.

There is no one acceptable approach to treat thin endometrium; largely due to the lack of a universally effective treatment. After ruling out medical and surgical risk factors for thin endometrium most physicians will refer their patients for sonohysterogram and a hysteroscopy. The advantages of hysteroscopy over a hysterosalpingram or a sonohysterogram include the ability to assess the vascularity of the endometrium and diagnose superficial adhesion as well as to perform adhesiolysis.

The following is a description of currently practiced strategies in treating thin endometrium. Some of the treatments might be of some efficacy at least for a certain sub-groups of patients.

Estrogen Administration

The successive use of estrogen and progesterone respectively is the adequate hormonal preparation for inducing endometrial receptivity [33].

Estrogen can be administered in several ways; the more popular being oral administration. It was shown that the effect of estrogen on endometrium is not through direct or regional transmission from the neighboring ovaries but rather through the systemic circulation [34]. External administration of estrogen may alter the concentration of serum and endometrial estrogen however; the impact varies according to the route of administration. Estrone (E1) is the least potent estrogen. Administering estrogen orally is more convenient however; estrogen undergoes first pass metabolism as well as metabolism in the endometrium resulting in stable estradiol (E2) levels in the serum but a higher E1 /E2 ratio [35]. Non oral routes of estrogen administration bypass the first phase of metabolism in the gastrointestinal tract. Vaginal estrogen is usually well absorbed and leads to similar concentrations of serum estrogen [36], however the endometrial concentration is much higher and the E1/E2 ratio is the lowest [37]. Usually the vaginal route is kept for unresponsive patients. Transdermal estrogen share the same advantage of avoiding first pass metabolism and it leads to more steady-state levels of serum estrogen [38] however, the

rate of absorption varies among patients but can be monitored by measuring serum levels [33].

For most patients an adequate endometrial response to estrogen can be achieved via any of the methods described above; and therefore, the simplest route preferred by the patient should be chosen. If the endometrial thickness goal is not reached, alternative routes should be considered as well as extending the length of the treatment [33].

In fresh cycles endometrial stimulation is a byproduct of ovarian stimulation that leads to the secretion of follicular estrogens. Therefore, most of the special protocols of estrogen administration for endometrial preparation were designed for frozen thawed embryo transfer cycles (FET). However, some of these protocols may be combined as part of the treatment of thin endometrium in IVF-fresh embryo transfers. Protocols differ in length and way of administration of estrogen and progesterone as well as the addition of adjuvant treatments.

Extended estrogen administration was described as a to overcome the problem of thin endometrium. Recently, Liu et al conducted a study trying to adjust estrogen administration according to estradiol serum levels, starting with 18 mg/day of estrogen Valerate and keeping the estradiol levels between 600-5000 pg/ml [39], 8 mm endometrial thickness was reached in 92.1%; the results of this group were comparable with the control group. The authors concluded that it is the duration of estrogen administration and not the serum concentration of estradiol that is critical to the success of the treatment [40].

Demir, et al. administered 4 mg estradiol hemihydratem for patients with thin endometrium, starting on triggering day in intracytoplasmic sperm injection cycles (ICSI), however no benefit was demonstrated [41].

Shen, et al. [42] published a case report describing treatment of a thin endometrium on a fresh IVF cycle by administering a high dose of (16 mg/day) of estrogen Valerate starting on day 3 and resulting in pregnancy.

A comparison between oral and vaginal administration of estrogen in FET cycles of blastocyst stage embryos for patients with an inadequate endometrial thickness, showed a longer duration of administration of estrogen and a higher dose with the vaginal route but a higher pregnancy rate [43].

Low Dose Aspirin

Steer et al. indicated in his study published in 1995 [44] that there is a link between uterine artery blood flow and endometrial receptivity. Several other studies demonstrated more favorable results in patients treated with low dose aspirin in ART cycles [45,46]. Two prospective randomized studies that were published in 1997 and 2000 [47,48] presented a higher implantation rate in patients with thin endometrium treated with low dose Aspirin 81 mg and 100 mg (respectively) without a significant increase in endometrial thickness. A Cochrane review in 2010 [49] found no benefit in adding aspirin for endometrial preparation.

Nitrates (Vitamin E, Sildenafil)

Vitamin E is considered antioxidant and scavenger of radical particles [50]. Cecik, et al. found that patients with unexplained infertility treated with vitamin E had significantly thicker

endometrium (mean of 9.6 vs. 8.2 mm) but no significantly different implantation or pregnancy rate [51].

A study of combination of vitamin E and Pentoxifylline demonstrated an improved endometrial thickness and pregnancy rate [52-54].

Takasaki, et al. conducted a study comparing several treatments for thin endometrium [55]. Vitamin E, L-arginine and Sildenafil citrate were shown to improve endometrial thickness and blood flow in 52%, 67% and 92% of the patients respectively.

Nitric oxide (NO) synthase has been identified in uterine blood vessels including the endometrium [56,57]. NO-cGMP pathway leads to the relaxation of vascular muscle system [58]. The inhibition effect of Sildenafil citrate (Viagra), a phosphodiesterase (PDE) inhibitor; stops the breakdown of the cGMP and enhances the NO-cGMP pathway and vascular muscle relaxation [59]. In 2000 preliminary study results were published demonstrating improved endometrial thickness and pattern as well as uterine blood flow; in 4 patients with recurrent IVF failure and endometrial thickness of <8 mm [60] following treatment with intra vaginal Sildenafil suppositories (25 mg 4 times a day). A case report of 2 women with Asherman syndrome treated successfully with Sildenafil [61] however, this positive clinical effect could not be repeated in latter studies.

A retrospective analysis of different adjuvant therapies during ART could not show beneficial effect of adding Sildenafil [62].

G-CSF- treatment

Normal endometrial decidualization is obligatory step for implantation to occur and several cytokines and growth factors were found to be involved in this process [63,64]. Triggering inflammatory reaction leads to increased secretion of these cytokines and growth factors most likely being the mediating factor behind the improved outcome following the endometrial scratching procedure. This procedure is usually offered to patients with recurrent implantation failure in order to improve pregnancy chances [65]. A similar effect is achieved by administration of Granulocyte colony stimulating factor (G-CSF).

G-CSF is a glycoprotein that combines growth factor and cytokine activities. It is secreted in various tissues, including reproductive tissues such as the endothelium and ovarian follicles as well as immunocytes like macrophages [66]. In 2011, a case report was published describing a new treatment administered to 4 patients with persistently thin endometrium. The patients received a single intrauterine wash with G-CSF 30 mU. In a latter publication, Gleicher, et al. reported on a series of 21 patients who were opted to be treated with G-CSF if their endometrium on the day of HCG triggering was < 7mm, if the endometrium did not thicken after 48 hours they received another G-CSF intrauterine wash at day of oocyte retrieval. The result was an average increase in endometrial thickness of 2.9 ± 1.9 mm and a pregnancy rate of 19.1% [67]. Similar low pregnancy rate was also observed in the prospective study conducted by Kunicki, et al. [68] the described a pregnancy rate of 18.9% despite an increase in endometrial thickness. Two other controlled trials; one of frozen embryo transfer (FET) cycles [69] and fresh IVF cycles [70]. These studies demonstrated no improvement in pregnancy rate and implantation rate in comparison with the control groups. Another prospective study conducted by Xu, et

al. [71] compared 2 protocols of G-CSF administration in FET cycles for patients with endometrium thickness <7 mm. The first protocol included G-CSF only and second included G-CSF and endometrial scratching. In both groups the endometrial thickness increased (from 5.7 ± 0.7 to 8.5 ± 2.4 and 8.3 ± 1.6 respectively) when comparing both groups to control untreated group of patients there was a significant increase in clinical pregnancy (48.1% vs. 25.0%) and a non-significant increase in live birth (33.3% vs. 17.3%).

HCG Injection

Papanikolaou, et al. in 2013 published an innovative concept of treatment of patients with recurrent implantation failure and persistently thin endometrium. The patients were treated with daily injection of hormonal chorionic gonadotropins (HCG) 150 IU in frozen embryo transfer cycles. They observed improvement in endometrial thickness of 20% or more in 35.3% of the patient and 17% achieved endometrial thickness of >7 mm [72].

Endometrial Progenitor Cells

Endometrial stem or progenitor cells have been identified and widely investigated. Endometrial mesenchymal cells (MSC's) own bone marrow MSC's features, they have regenerative potential and are easily obtained from endometrium.

Bone Marrow Stem cells

Regenerative medicine evolved in the last few years in different medical disciplines, Stem cells as a source for regenerating different tissues had been widely investigated.

Jing, et al. [73] published a study using a rat model of thin endometrium treated with bone marrow stem cells (BMSC's) for thin endometrium. In this study BMSC's, were administered intra-venous (IV), the results were favorable; endometrium has thickened and better receptivity associated factors were differentially expressed after the treatment.

A randomized control study conducted by Zhao et al demonstrated good endometrial response in rats; after intrauterine injection of BMSC's [74]. The assumption is that the migration and the immunomodulation effect of the BMSC's causes regeneration of the abnormally thin endometrium [73,74]. Further studies are needed to establish this treatment as an option for human in terms of safety and efficacy.

Mesenchymal Progenitor Cells

Recently, native endometrial progenitor stem cells were identified [75-77]. These cells are of a mesenchymal origin. These types of progenitor cells were, also, identified in the adipose tissue [78], these cells have the ability to differentiate into various cell types and are categorized as stromal vascular fraction (SVF). It is relatively easy to extract these cells from the adipose tissue and they contain a mix of cell subtypes including: endothelial, perivascular, fibroblast immune and mesenchymal [79]. A study conducted on a rodent model of thin endometrium, could not demonstrate any positive effect after intrauterine administration of these cells.

Another cell type is stromal fibroblasts; they can be retrieved from endometrium or menstrual blood. These cells have shown multi-lineage differentiation potential [80]. Other endometrial

cell types with stem cells properties have been described opening new windows for regenerative treatment of endometrial disorders including thin endometrium. However, these cells should investigate further to improve our understanding of their function and safety in order to allow future clinical use.

Conclusions

Persistent thin endometrium remains one of the most challenging disorders in reproductive medicine. No definite treatment has yet been established, and the options are limited and are not problem specific. Though extended estrogen regimens are the easiest to offer, they do not provide a solution for a big portion of these patients. Other treatments options: low dose aspirin, nitrates, GCSF and HCG lack convincing evidence of utility for treating thin endometrium and more comparative studies should be conducted to prove and perhaps improve these options as well as identifying the specific sub group of patients to which some of these treatments might be more effective. Regenerative medicine is a promising new treatment modality that is currently undergoing an intense research including in reproductive medicine; initial studies demonstrate positive results of endometrial thickness improvement but there is still a long way till these treatments may become clinically applicable.

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