Review

Research on Recent Progress in the Treatment of Thin Endometrium

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Abstract: The endometrium is an important part of the uterus. The human endometrium is a complex and dynamic tissue that goes through phases of growth and regression during any menstrual cycle. A thin endometrium might be relevant to a lower rate of implantation as well as a higher rate of miscarriage. Several treatments have been developed for thin endometrium, such as granulocyte colony-stimulating factor, stem cell therapy, acupuncture and physical therapy, among others. These approaches have been shown to have effects on the endometrium related to reducing the area of fibrosis, increasing the number of glands, promoting angiogenesis, increasing endometrial thickness, improving tissue structure, and increasing pregnancy rates. This review summarizes the key role of these treatments in repairing thin endometrium and improving clinical pregnancy rates

Keywords: Thin Endometrium; stem cells; Granulocyte colony-stimulating factor; Acupuncture treatment; Physical therapy

1. Introduction

Successful implantation of the embryo requires appropriate embryonic development and also requires that the mother has a well-conditioned endometrium. In humans, the uterus becomes receptive to implantation in the mid-luteal stage of the menstrual cycle, being often called as the window of implantation. Implantation is a complicated course that is subject to complex molecular regulatory mechanisms. (Fig. 1) The original step of the implantation course is the juxtaposition and contact of the blastocyst with the endometrial epithelium, and then it is the invasive activity of trophoblast cells between the epithelial cells. It has been reported in the literature that inadequate tolerance of the uterus will lead to about half of the implantations are abnormal and pregnancy failure [1-3]. Although assisted reproductive technologies have helped humans overcome most infertility problems, successful embryo implantation remains an important step for successful in vitro fertilization.

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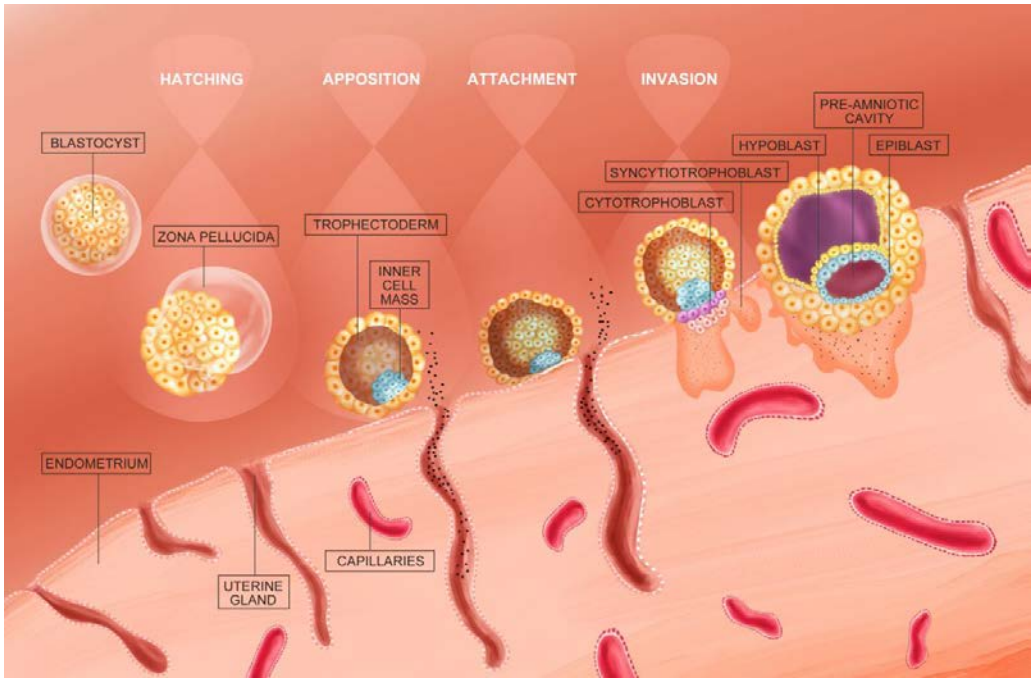

Figure 1. Embryo implantation process under normal physiological conditions.

The endometrium consists of two cellular components, that is to say, surface epithelial cells and mesenchymal cells. The endometrium is not stable and unchanging, but is a actional tissue going through several changes in the period of the menstrual cycle consisting of morphological histology, biochemistry and molecular biology, essential elements for a successful embryo implantation course. Estrogen and progesterone stimulate the endometrium, with estrogen causing diffusion and incrassation of the endometrium, and progesterone causing excretive variation, metaplasia, puffiness and thickening of the endometrium. These two hormones at the same time can promote the development of the endometrium and cause variation of endometrial mesenchymal cells into metaphase, ultimately providing an acceptable situation for implantation of the blastocyst.

In assisted reproductive techniques, the growth of the mother's endometrium is usually under observation by transvaginal ultra audible sound. An accepted marker of uterine capacitance is the thickness of the endometrium [4]. For successful embryo transfer, the recommended minimum endometrial thickness is 7 mm, be it that there is no standard value for diacrisis of a thin endometrium [5]. Additionally, higher implantation rates can be achieved with endometrial thicknesses greater than 9 mm. In contrast, a persistently thinner endometrium may be associated with a lower rate of implantation as well as a higher rate of miscarriage [6]. Therefore, many researchers around the world have tried to find some treatments that can improve endometrial tolerance and endometrial thickness. Although several treatments have been tried clinically, clinical outcomes have been inconclusive. In this review, we review the literature on thin endometrium and discuss recent therapeutic tools and recent progress of thin endometrium to inform clinicians on better treatment options.

2. Granulocyte Colony-Stimulating Factor（G-CSF）

G-CSF is a hemopoietic development factor, having positive influence on non-hematopoietic cells, including the endometrium [7]. In an expected cohort research conducted by Gleicher et al. in 2011, the authors' team used G-CSF for transvaginal endometrial perfusion to treat thin endometrium. The results showed that four patients previously resistant to estrogen and vasodilator therapy had triumphant endometrial inflation to a minimum concentration of at least 7 mm in the back of uterine perfusion with G-CSF, sug-

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gesting intrauterine G-CSF might impose direct influence in promoting endometrial development [8]. A preliminary cohort research [9] showed an increase in endometrial ply from 6.4 mm to 9.3 mm at an interval of 5.2 days between G-CSF infusion and embryo transfer. The mean variation was 2.9 mm and did not change between pregnancy and non-pregnancy circles. This is sufficient to demonstrate the practical use of G-CSF in the therapy of chronic thin endometrium and that this therapy would lead to a good overall clinical pregnancy rate (19.1%). Lucena et al[10] discovered uterine filling of G-CSF rapidly enhanced the ply of the endometrium, thus ensuring a higher chance of successful pregnancy and a healthy birth ratio of the baby. The outcomes suggest G-CSF is an element involved in endometrial fashioned, which enhances the hold-in between the uterine circumstance and embryonic growth.

Li et al [11] estimated the effectiveness of G-CSF in a frozen embryo diversion program for infertile women having thin endometrium. The G-CSF group had a higher rate of entice circles and a lower rate of natural circles compared to the control group and showed a tendency towards better infusion and clinical conception ratios. Equally, Kunicki et al. reached similar conclusions [12]. Kunicki's team did a follow-up visit of 37 subjects with thin and unresponsive endometrium on the day of ovulation producing. In all subjects, endometrial ply was 6.74 mm before G-CSF injection and enhanced greatly to 8.42 mm after infusion [12]. In another non-randomized interventional clinical experiment, Eftekhar et al [13] compared the effects of in utero G-CSF treatment and immediate embryo shift in suffers with endometrium (<7 mm). All suffers were treated with oral oestrogen and transvaginal sildenafil and on day 12 or 13, patients in the G-CSF group would receive intrauterine G-CSF therapy. While this research failed to prove the possibility of G-CSF to improve endometrial thickness, it is possible to use G-CSF treatment to enhance clinical conception rates in barren women having thin endometrium in frozen-thaw embryo shift circles. Tehraninejad et al[14] performed intrauterine G-CSF infusion in 15 patients who underwent embryo transfer and were recalled due to thin endometrium. The endometrial thickness of these patients increased from 3.59 mm to 7.120 mm with a clinical conception rate of 20%.

However, negative outcomes were also obtained by some research teams. Barad et al [15] conducted a stochastic parallel double-blind controlled clinical experiment to determine whether G-CSG influences endometrial ply, infusion rate and clinical conception rate. The results showed there is no statistic difference in increased endometrial thickness between the G-CSF group and the experimental group. However, this study was acquired in an elder suffer population, so they might not work for younger women. In another prospective study, Miralaei et al [16] found a remarkable change in endometrial ply after G-CSF therapy ($p<0.001$); however, nine patients (45%) did not reach an endometrial ply of 7 mm and therefore the embryo shift was cancelled. The above evidence allows people to know that although intrauterine infusion of G-CSF has a possibility in increasing endometrial ply in sufffers, the rate of transfer failure remains high and events of poor conception outcome are under observation.

In 2016, Lee and his team explored the effectiveness of intrauterine infusion of G-CSF in infertile females with thin endometrium on trigger day or the day of egg retrieval [17]. The general clinical conception rate was 22.0%, the infusion rate was 15.9%, and the unremitting conception rate was 20%. Intriguingly, a tendency appears towards higher infusion, clinical conception rates and sustained conception rates with G-CSF infusion on the trigger day [17]. This provides a clinical rationale for the timing of intrauterine G-CSF infusion.

3. Stem cell treatment

Among many types of cellular therapies, stem cell therapy is considered to be an effective treatment [18]. Stem cells have the ability of differentiating into pluripotent stem cells, and several studies have been conducted to enumerate the advantages and disadvantages associated with stem cell therapy [19]. A recent review published in the journal

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Cell in 2021 writes that stem cells are now increasingly considered as promising alternative therapies for translational research in regenerative medicine. Considering the less ethical issues and easy access to abundant resources, induced pluripotent stem cells and mesenchymal stem cells have been extensively studied within the field of infertility to understand their potential applications in reproductive medicine. (Stem cell classification table)

Similar to most treatments, stem cell therapy can come with side effects. For example, before stem cells are used, they are harvested from embryos and cultured for several months. When stem cells are harvested from the adult body, especially from the bone marrow, it can put the patient through many painful procedures. In addition, stem cell treatment has the great possibility to cause a certain percentage of rejection [20].

3.1. Mesenchymal Stem Cells

MSCs are adult stem cells which could be obtained from various tissues, consisting of bone marrow, umbilical cords, menstrual blood, endometrial tissue, and adipose tissue. Given their competence in self-renewing and differentiation, MSCs are considered by some studies to be the most attractive cell therapy candidates in regenerative medicine [21]. This property can reflect the origin of the tissue, as MSCs isolated from different tissues show different sensitivities to inducible bioactive molecules in the culture medium. A well-known example is adult bone marrow-derived MSCs, the normal category of MSCs. The circumstances of induction of bone marrow-derived MSCs are different from those of adipose-derived MSCs, which may be explained by the existence of a different microenvironment in the vascular system where the cells are located [22]. In addition, several in vitro experiments have demonstrated the excellent in vitro regenerative potential of MSCs. The protective role played by bone marrow MSCs after allogeneic implantation has been said in couple types of injury [23].

It is currently believed the remedial influences of MSCs are chiefly because of their immunomodulatory function relevant to anti-inflammatory influences via regulating the accommodative and inherent immune system lymphocytes. In addition, MSCs have been shown to modulate the immune response in a variety of diseases [24]. Besides, MSCs are able to regulate T cell function and proliferation, balance Th2 and Th1 activity, upregulate Tregs function, inhibit B and NK cell function, and prevent dendritic cell activation and maturation [25]. Additionally, MSCs stimulate the proliferation and cytokine secretion of innate lymph-like cells, a new family of lymph-like cells that take a great part in innate defense against pathogens.

The effectiveness of MSCs in treating thin endometrium has also been confirmed by several studies. Zhao et al [26] established a rat type having thin endometrium by injecting ethanol into the uterine cavity so as to inquire into whether direct implantation of MSCs into the uterine cavity could improve endometrial thickness. The outcomes displayed that the endometrium of rats in the intrauterine cavity transplanted with MSCs group was significantly thickened, and the expression of cytokeratin, wave protein, integral protein $\alpha\gamma\beta3$ and leukemia inhibitory element was higher than that of the experiment group. The utterance of certain cytokines leading to inflammation was greatly downregulated, while the expression of anti-inflammatory cytokines was significantly upregulated. The authors concluded that uterine perfusion of MSCs stands for a hopeful novel remedial implement to address the presently tricky issue of endometrial thinning. In another study, Jing and his team explored whether bone marrow MSC treatment could promote endometrial regeneration and improve endometrial tolerance [27]. They implemented a randomized controlled animal study in which bone marrow MSC transplantation was performed by tail vein injection. The outcomes displayed the endometrium of the control group was greatly thickened and the expression of cytokeratin, wave protein, integrin $\alpha\gamma\beta3$, and leukemia inhibitory factor was significantly enhanced compared to the control group. The above evidence could suggest that MSCs are beneficial to thin endometrium, which may act through the migration and immunomodulation of MSCs.

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3.2. Mesenchymal Stem Cell-derived Extracellular Vesicles (MSC-EVS)

Latest researches have also concentrated on the study of exosomes excreted by mesenchymal stem cells. Exosomes are effective paracrine elements having a great possibility to repair destroyed tissues. Exosomes consist of a lot of paracrine elements accountable for reconstruction and vasculogenesis [28].MSC-EVS is a lipid bilayer complex that acts as a mediator by transferring multiple molecules (e.g., proteins, microRNAs, lipids, and cytokines) to the recipient cells. The basic mechanisms of action of MSC-EVS have been agreed upon and include promotion of vasculogenesis, anti- fibering, immunomodulation and antioxidant pressure standards.

However, many issues have to be totally substantiated before MSC-EVS can be used in the clinic, consisting of standardized purification and identification methods, applicable storage and transport systems, large scale production facilities, and safety issues. Additionally, limited output is one of the major issues limiting the widespread use of MSC-EVS. Overall, MSC-EVS shows great potential in regenerative medicine compared to MSCs, not only because it is derived from parent cells, and it has high-level biological consistency and low-level immunogenicity [29].

3.3. Human Amniotic Epithelial Cells (hAECs)

As a possible source of stem cells, hAECs are extracted from the amniotic membrane, which contacted the amniotic fluid and also is the layer of tissue closest to the foetus. Many studies have reported the immune adjustment effects of hAECs on acquired immune cells and geneogenous immune cells. In addition, hAECs can differentiate into many cells of mesodermal and ectodermal origin, consisting of neuronal cells, pancreatic cells, hepatocytes, adipocytes, myocardial cells and myocytes. Besides, hAECs can inhibit the B cells proliferation and suppress the transfer and proliferation of neutrophils and macrophages [30]. Additionally, hAECs inhibited the liveness of CD4+ T cells and reduced the generation of pro-inflammatory cytokines by CD4+ T cells. According to the literature, hAECs significantly enhance proliferating cell nuclear antigen, which is essential for precise DNA replication [31]. Punyadeera et al [32] analyzed the mRNA expression levels of all known vascular endothelial growth factor ligands and receptors in human endometrium collected during the menstrual period and proliferative course of the menstrual period. The results showed that PCNA was most abundant in both epithelial and mesenchymal tissues in the proliferative phase. However, the expression of hAECs was decreased in the endometrium of mice during the secretory phase, suggesting that hAECs may have a role in promoting endometrial proliferation. Vascular endothelial growth factor was mainly expressed during the proliferative and menstrual phases, which is associated with the maintenance and formation of micro vessels and the reconstruction of endometrial tissue.

Chen et al [33] verified that intrauterine adhesion release combined with hormone replacement therapy significantly increased endometrial vascular endothelial growth factor expression and microvascular density in subjects with severe uterine adhesions. Besides, patients with better outcome did have more VEGF expression and denser microvasculature compared to those with poor treatment response. hAECs were found to increase VEGF expression in a model of intrauterine adhesions by Zhou et al [34], suggesting that hAECs have the potential to promote angiogenesis in the injured endometrium. The estrogen receptor, a nuclear transcription factor, binds to estrogen to promote endometrial cell proliferation and metabolism. In one study, umbilical cord-derived mesenchymal stromal cells were added onto a collagen scaffold and implanted into the womb after a uterine adhesion separation operation [35]. 3 months later after surgery, patients had an increase in mean maximum endometrial thickness and a decrease in uterine adhesion scores compared to pre-treatment. Histological studies showed upregulated levels of estrogen receptor, wave protein and vascular hemophilia factor expression, suggesting

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improved endometrial proliferation, differentiation and neovascularization after treatment. Another study came to a similar conclusion that hAECs significantly improved the uterine architecture after uterine adhesions. hAECs treatment resulted in thickened endometrium, increased number of endometrial glands, and reduced fibrosis, which in turn produced more micro vessels. Expression levels of vascular endothelial growth factor, PCNA and estrogen receptor were increased in hAECs-treated endometrium, indicating improved angiogenesis and stromal cell proliferation. Finally, hAECs also increased pregnancy outcome, pregnancy rate and fetal number in mice with uterine adhesions [36].

4. Acupuncture and physical therapy

Acupuncture is a representative of traditional Chinese medicine and has accumulated much clinical experience in the treatment of gynecologic infertility. For thin endometrium, acupuncture therapy has shown the same great potential for clinical application. Acupuncture is an important part of traditional Chinese medicine, which applies mechanical stimulation through the use of needles at specific acupuncture points, thereby regulating the body's functions.

Several studies have shown that acupuncture treatment for patients can actually increase the clinical pregnancy rate and improve ovarian-uterine blood flow at embryo implantation [37]. Performing transcutaneous acupoint electrical stimulation during the preimplantation phase can promote increased expression of endometrial angiogenesis and stromal cell proliferation-related factors, resulting in a significant improvement in endometrial tolerance [38]. Mechanistic studies have shown that progesterone levels were significantly increased after transcutaneous acupoint electrical stimulation treatment, and integrin family proteins and leukemia inhibitory factors were significantly increased and positively correlated with the increase in progesterone. Li Yu et al. adopted randomized method to grouped 90 subjects who failed to conceive due to unexplained endometrial dysplasia into two parts by randomization. The experimental group was applied with transcutaneous electrical acupoint stimulation from the 5th day of menstruation, supported by progesterone after ovulation and embryo transfer three days after ovulation; the observation group was given conventional estradiol valerate and progesterone support. The outcomes proved that the pre-transfer size and endometrial kind improved remarkably in the experimental group in comparison to the observation group. Among the subendometrial blood flow parameters, the resistance index and fluctuation index of the experimental group were significantly lower than those of the observation group, which means it has statistical significance ($P<0.05$). In terms of clinical pregnancy rate, the experimental group was higher than the observation group, and the result has statistical significance.

Zhang et al [39] evaluated the function of transcutaneous electrical acupoint stimulation on pregnancy rates. This was a prospective, randomized, single-blind and placebo-controlled clinical research involving subjects who went through cryopreserved embryo transfer or fresh cycle external fertilization with or without intracytoplasmic single sperm injection. The results showed that one day before transplantation, administration of transcutaneous electrical stimulation of acupuncture points for 30 min increased clinical pregnancy rate by 13%, and administration of 2 transcutaneous electrical stimulation of acupuncture points 1 day before transplantation and 2 times after transplantation increased clinical pregnancy rate by 20%. Low-frequency electrical stimulation at 2 Hz was superior to high-frequency electrical stimulation at 100 Hz, and acupuncture points on the abdominal dorsum (Gui Lai, Zi Gong, Guan Yuan, and Shen Yu) were more beneficial than acupuncture points on the extremities (Xue Hai, Di Ji, Zu San Li, and Tai Xi) in accordance with fertility and clinical conception rate [39]. Meng Qingyu et al [40] observed the effect of electroacupuncture combined with bone marrow mesenchymal stem cell injection on estrogen and progesterone receptors in thin endometrium of rats. The outcomes proved that the uterine coefficient and the expression of Ki67, estrogen receptor, and progesterone receptor were significantly higher in the electroacupuncture combined with

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bone marrow MSC injection group in comparison to the observation group, and this effect was superior to that of bone marrow MSC injection alone. Similar conclusions were reached by You et al. who found that high-frequency electroacupuncture was effective for improvement blastocyst implantation in rats with impaired endometrial tolerance. High-frequency electroacupuncture clearly increased endometrial size and counts of pinnae. This effect may be achieved by enhancing the LIF/STAT3 signaling pathway in mice [41]. The above evidence demonstrates that acupuncture therapy promotes endometrial growth, improves endometrial tolerance, and ultimately promotes embryo implantation and clinical pregnancy.

Several physical therapies including pulsed electromagnetic fields and massage have also had a positive impact on improving endometrial tolerance and increasing endometrial thickness. Merhi Z et al. combined transcutaneous ozone therapy with pulsed electromagnetic field therapy to treat patients with thin endometrium. The results showed that ozone with pulsed EMF therapy had vasodilatory, anti-inflammatory and antioxidant effects and successfully improved endometrial lining thickness in all patients, with two-thirds of patients becoming pregnant after single embryo transfer [42]. Yang et al. investigated the function of pelvic floor neuromuscular electrical stimulation treatment for the improvement thickness of endometrium. The mean endometrial thickness before and after the experimental group was 5.60 mm and 7.93 mm, separately, compared with the values in the observation group, with statistically remarkable differences, so neuromuscular electrical stimulation treatment may be practicable in patients with thin endometrium [43]. Electro-ultrasound is a new type of physical therapy currently available, which combines transcutaneous acupoint electrical stimulation technique, pelvic floor neuromuscular electrical stimulation technique and acupoint ultrasound technique. Acupuncture point ultrasound is a new acupuncture technique that uses the technique of ultrasound to simulate acupuncture to achieve a non-invasive acupuncture effect. (Photo presentation) Zhang et al. [44]2021 studied 80 subjects with thin endometrial infertility, in which 40 patients in the observation group were applied with estradiol valerate and 40 subjects in the observation group were applied with estradiol valerate in combination with electro-ultrasound. The results indicated that the endometrial thickness, morphology and volume improved in both groups, and the difference was more obvious in the observation group ($P<0.05$). After treatment, the uterine artery resistance index and type I flow ratio were significantly reduced and type III flow ratio was clearly increased in the observation group, and the endometrial and subendometrial vascularization index (VI), blood flow index (FI) and vascularized flow index were clearly improved in the observation group, then the differences had statistical significance ($P<0.001$). The clinical pregnancy rate in the observation group was higher than that in the observation group, and the result has statistical significance ($P<0.01$). In addition, a research found that the massage of pelvic floor muscles can efficiently increase the clinical pregnant rate. The theory is to stimulate muscular contraction, raise Internal abdominal pressure, improve pelvic blood circulation, decrease uterine artery blood circulation resistance, and raise subendometrial blood circulation and perfusion, which improves the size and form of the endometrium and improves the positive function and hormonal levels of the uterus and ovaries [45].

5. Conclusion

As a common illness, infertility acts on a mass of women and is a public social medical problem. In view of the important role of the endometrium in maternity health and propagation, it is crucial to maintain the physiological function, eliminate the faults and promptly recover after damage. Several treatments have been developed, such as granulocyte colony-stimulating factor, sildenafil, small dose aspirin, stem cell therapy, acupuncture and physical therapy, among others. Overall, some of the newer therapies, including granulocyte colony-stimulating factor and stem cell treatment, may enjoy many advantages over conventional therapies. However, there is still room for improvement in these therapies and many studies are needed to amplify the potential of new treatments.

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In the end, as the feasibility of acupuncture and physical therapy for thin endometrium is proven, these therapies should likewise receive adequate attention.

Authors' contributions

XT and KN wrote this manuscript; XT prepared all the figures and optimized the language; KN designed the structure and edited the manuscript; all authors have reviewed and agreed the final version.

Competing interests

The authors announced that there is no conflict of interest among them.

Declarations

All authors announced they have no actual or potential conflict of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have agreed to publication.

Availability of data and materials

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