ingegneria sismica
International Journal of Earthquake Engineering
Anno XLIII - Num. 3 - 2026

Progress in research on immune direction of thin endometrium

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SUMMARY: *Thin endometrium, as a core element affecting the success rate of embryo implantation and overall pregnancy rate, has always been a research hotspot in the field of reproductive medicine. This article systematically reviews the latest research trends on the immunological aspects of thin endometrium, with a focus on analyzing the pathways of immune related mechanisms in its pathogenesis, and comprehensively summarizes current feasible intervention strategies, in order to provide scientific guidance for clinical practice. This article first clarifies the concept definition, clinical characteristics, and pathogenic factors of thin endometrium, and deeply analyzes its interaction with key immune cells such as macrophages, T lymphocytes, and NK cells. Meanwhile, the system elucidated the regulatory roles of cytokines such as granulocyte colony-stimulating factor (G-CSF), vascular endothelial growth factor (VEGF), leukemia inhibitory factor (LIF), etc. in the process of endometrial remodeling. In addition, this article systematically introduces the progress of immune regulation therapy, covering traditional drug interventions and new technologies in regenerative medicine. Existing studies have shown that immune system dysfunction can affect endometrial receptivity through various pathways, including Th1/Th2 cytokine imbalance, excessive activation of natural killer cells, abnormal expression of human leukocyte antigens, and regulatory T cell dysfunction. These mechanisms may directly or indirectly lead to insufficient endometrial thickness, ultimately hindering normal embryo implantation. The complex regulatory network composed of immune cells and cytokines plays a key role in the pathogenesis of thin endometrium. In terms of clinical intervention, traditional treatment methods such as estrogen replacement therapy, growth hormone supplementation, pelvic neuromuscular electrical stimulation, as well as regenerative medicine techniques such as local injection of platelet rich plasma, targeted delivery of growth factors, mesenchymal stem cell transplantation, and G-CSF intrauterine perfusion, have all shown varying degrees of improvement effects. These research advances will provide important theoretical support and technical guarantees for improving the reproductive health level of patients with thin endometrium.*

KEYWORDS: *Thin endometrium; Macrophages; T lymphocytes; Immune cells; Pregnancy; Macrophage*

1 Introduction

In the vast field of medical science, there is still a clear lack of recognized and strictly standardized definitions for thin endometrium, causing significant gaps in clinical and research paradigms. Although there is no clear diagnostic threshold, numerous studies have carefully documented a strong and convincing association between endometrial lining with a thickness

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https://doi.org/10.65102/is20261177
Published: 10 June , 2026

less than 7 millimeters and a significantly reduced likelihood of achieving successful pregnancy outcomes [1]. This phenomenon has received consistent attention in different patient populations and research environments, greatly increasing academic attention, guiding people to work hard to decipher the complex pathogenic mechanisms of this disease, and research feasible treatment methods. However, despite these collective efforts, many aspects of the fundamental pathophysiology of endometrial thinning remain unclear, and there are significant obstacles in designing effective treatment methods.

The causes behind the thinning of the endometrium are extremely complex and involve numerous potential factors. Among them, the recognized hormonal imbalance is an important aspect, which may disrupt the delicate balance of the endocrine environment necessary for optimal proliferation and differentiation of the endometrium; In addition, vascular dysfunction cannot be ignored as it hinders the delivery of key nutrients and oxygen to the endometrial tissue. These factors are intertwined and influence each other, and their complex nature makes it difficult to accurately identify a single pathogenic factor, thereby making the diagnosis and treatment process more complex [2].

Faced with such complex causes, there is currently no unified consensus on the effectiveness of various treatment methods for thin endometrium. Clinical doctors usually adopt a series of intervention measures, which have varying effects and are based on different evidence bases. For example, hormone therapy, especially estrogen replacement therapy, is a commonly used method for treating thin endometrium and one of the most widely used treatment strategies [3, 4]. The basic principle of this therapy is that estrogen plays a crucial role in promoting endometrial growth and enhancing its ability to accept embryo implantation. However, despite the widespread use of estrogen therapy, the therapeutic effects vary greatly, highlighting the importance of developing personalized treatment plans that fully consider the specific circumstances and individual responses of each patient. In addition to estrogen, other hormone drugs have also received research attention due to their potential to increase endometrial thickness. For example, human chorionic gonadotropin and growth hormone have been explored in various clinical settings, but the results vary due to differences in patient populations and treatment plans [5]. In addition to hormone interventions, strategies aimed at improving endometrial blood flow are gradually gaining recognition. Low dose aspirin is a commonly used clinical medication, which is believed to improve uterine blood perfusion by inhibiting platelet aggregation and promoting vasodilation, potentially increasing endometrial thickness and enhancing its receptivity.

In addition, the rise of regenerative medicine has opened up new avenues for the treatment of thin endometrium. Preclinical studies and early clinical trials have shown that granulocyte colony-stimulating factor and platelet rich plasma have potential value in stimulating endometrial regeneration and improving reproductive outcomes. These regeneration technologies utilize the body's own healing mechanisms to promote tissue repair and regeneration, providing a novel and relatively less invasive alternative to traditional treatment methods. It is worth noting that recent evidence has begun to reveal the possible immunological basis for endometrial thinning, suggesting that immune system dysfunction may play a key role in the pathogenesis of diseases. This emerging viewpoint emphasizes the importance of incorporating immune related factors into the diagnosis and treatment system of thin endometrium. Given the above developments, this review aims to provide a comprehensive and in-depth analysis of thin endometrium, with a particular focus on exploring the immune related factors that may lead to its occurrence, and sorting out the treatment options currently available to clinical doctors, in order to provide strong basis for clinical decision-making and improve the quality of patient care.

2 Overview of thin endometrium

2.1 Definition of thin endometrium

In the field of reproductive medicine in China, top experts in the industry are gradually forming a unified understanding of the clinical evaluation of endometrial health status. More importantly, these authoritative data indicate that when conducting diagnostic assessments, if the thickness of the endometrial lining is less than 7 millimeters, it should be recognized as a key criterion for determining thin endometrium [6]. Although this standard has not yet achieved unified standardization in all medical disciplines worldwide, it provides valuable reference for Chinese clinical doctors in diagnosis and treatment decision-making. The relevant investigations conducted by Zhao Jing and her research team have greatly deepened our understanding of the complexity and multidimensionality of endometrial assessment. Their research emphasizes that when evaluating the endometrium, it is not enough to simply measure its thickness, but a comprehensive evaluation strategy should be adopted. Specifically, it is important to pay attention to the evaluation of the morphological characteristics of the endometrium, including its structural integrity and cellular composition. In addition, the evaluation of uterine hemodynamics is crucial because only by ensuring sufficient blood perfusion can the endometrium function optimally and the embryo obtain necessary oxygen and nutrients.

Not only that, Zhao Jing's research also focuses on the complex relationship between endometrial health and the overall health status of patients, as well as the quality issues of embryos used in assisted reproductive technology (ART). This holistic perspective recognizes that the success of pregnancy depends on a delicate balance between multiple factors, including endometrial receptivity, embryo survival rate, and maternal health status. The natural fluctuation of endometrial thickness during the menstrual cycle is a widely recognized physiological phenomenon. However, if the thickness of the endometrium continues and gradually becomes thinner, it may be an ominous sign of decreased embryo implantation rate and increased risk of miscarriage. Numerous studies have confirmed this viewpoint, generally indicating a negative correlation between endometrial thinning and pregnancy success rate. Specifically, as the endometrium gradually thins, the probability of successful pregnancy typically decreases significantly, whether through fresh embryo transfer or frozen embryo transfer.

These research conclusions have profound implications for clinical practice, emphasizing the need for clinicians to adopt proactive and multidimensional approaches to evaluate and manage the endometrium during assisted reproductive therapy. By comprehensively evaluating the morphology and blood flow of the endometrium, as well as the health status of the patient and embryo, clinical doctors can improve their ability to predict pregnancy outcomes and adjust treatment strategies accordingly. Ultimately, this method helps to increase the chances of successful conception and the birth of healthy live births.

2.2 Characteristics of thin endometrium

The clinical manifestations associated with thinning of the endometrium cover a series of pathological and physiological changes, which work together to disrupt the receptivity of the endometrium, leading to a decrease in reproductive success rate. One significant manifestation is an increase in uterine artery resistance, which hinders the optimal delivery of blood to endometrial tissue. Such vascular dysfunction is often further exacerbated by damage to the proliferation of the upper cortex. As a key component of the endometrial structure, the epithelial layer plays an extremely important role in embryo adhesion and implantation. At the same time, the production of vascular endothelial growth factor (VEGF) significantly decreased. VEGF,

as a key angiogenic factor, plays an indispensable role in promoting angiogenesis and maintaining vascular homeostasis within the endometrium.

In addition to the functional deficiencies mentioned above, the typical characteristics of thin endometrium are also reflected in abnormal vascular morphology. The growth pattern of endometrial blood vessels often deviates from the normal state, presenting a sparse vascular network and decreased cell density. From an histological perspective, the irregularity of these blood vessels manifests as changes in cellular morphology. The nucleus and cytoplasm of endometrial cells usually show a decrease in staining intensity, which is a sign of reduced cell activity and metabolic function. In addition, spiral arteries may have significant defects or discontinuities, as they are responsible for delivering oxygen and nutrients to the functional layer of the endometrium during the luteal phase of the menstrual cycle.

In addition to these local changes in the endometrium, the clinical consequences of thin endometrium can also extend to various adverse pregnancy outcomes. Numerous research data have confirmed a close correlation between endometrial thinning and an increased risk of miscarriage. As a destructive complication, miscarriage has a significant impact on early pregnancy. Moreover, women with thin endometrium have a higher probability of developing gestational hypertension, which poses a serious threat to the health of both mother and baby. Another issue that cannot be ignored is the increased possibility of premature birth, which may lead to a series of neonatal complications related to premature birth.

Placenta previa is a disease characterized by abnormal implantation of the placenta above or near the cervical opening, and is more common in women with thin endometrium. This anatomical abnormality may lead to severe bleeding during delivery and childbirth, requiring emergency cesarean section (Caesarean section). In fact, when the endometrium is thin, the demand for cesarean section in pregnant women who undergo assisted reproductive procedures significantly increases, further highlighting the clinical relevance of this situation [7].

2.3 Causes of thin endometrium

The suboptimal endometrial lining that poses significant challenges to reproductive success typically stems from one or more of three basic categories: structural abnormalities, physiological damage, or unexplained (idiopathic). Each of these categories contains a range of potential mechanisms that may independently or synergistically lead to endometrial defects. In structural abnormalities, the inflammatory process is the main factor. Chronic or acute inflammation in the uterine cavity can damage the fine structure of the endometrium, leading to fibrosis, scar formation, and vascular damage. These structural changes are particularly evident after medical interventions such as dilation and curettage (D&C) surgery, and although sometimes necessary, may inadvertently damage the complex vascular network and glandular structure necessary for endometrial proliferation and differentiation. This type of condition can seriously weaken the ability of the endometrium to promote embryo implantation and ensure the smooth progress of subsequent pregnancies by disrupting normal blood perfusion and glandular activity.

Another important mechanism for defects in the endometrium is the disruption of its physiological functions. This disorder may manifest as hormonal imbalance, obstruction of intercellular information transmission, or poor decidualization ability of endometrial stromal cells, which is a key link in constructing a suitable endometrial environment for embryo implantation. The root cause of this functional defect may be systemic diseases such as polycystic ovary syndrome (PCOS) and thyroid disease, or it may be limited to the endometrium itself, reflecting inherent cellular or molecular abnormalities. In some cases, endometrial defects may be related to genetic susceptibility factors, as genetic variations can make individuals more susceptible to endometrial development or dysfunction. However, there

are still a considerable number of cases whose causes cannot be determined medically, which fully highlights the complexity and multifactorial characteristics of the disease [8].

3 Relationship between immune cells and thin endometrium

3.1 Relationship between macrophages and thin endometrium

Due to the unclear pathogenesis, the early diagnosis and treatment of thin endometrium have reached a bottleneck. The latest research shows that the abnormal colonization of endometrial tissue in the pelvic and abdominal cavity is closely related to immune surveillance disorders, so these "foreign substances" can be avoided from being cleared. The continued existence of endometrial tissue promotes oxidative stress and inflammatory response, and many inflammatory and growth factors in the pelvic and abdominal immune microenvironment are stimulated to maintain the lesions of endometrial tissue and cause organ damage.

Macrophages possess multiple effects such as antigen presentation, phagocytosis, secretion of inflammatory factors, and processing of old red blood cells, and are important members of innate immunity. Macrophages exhibit different immune regulatory functions through different polarization states. The function of macrophages plays a crucial regulatory role in the occurrence and development of endometriosis in the abdominal immune microenvironment. Previous studies have shown that macrophages are the sharp blades that specifically recognize and clear thin endometrial cells, and are a new carrier for immunotherapy [9]. In recent years, the use of modified immune cells for the treatment of tumors and other related diseases has become a trend, and there is even a high possibility of curing some diseases, so it is highly anticipated. There is recruitment and activation of macrophages at the lesion site of thin endometrial disease, mainly in the M2 polarization state. Interestingly, M2 macrophages at the lesion site of thin endometrial disease do not promote apoptosis of thin endometrial cells, but rather promote their proliferation; Similarly, peritoneal macrophages in thin endometriosis are more inclined towards M2 polarization and can secrete some cytokines that may impair fallopian tube function and early embryonic development. However, the polarization changes of peritoneal macrophages in thin endometriosis lead to abnormal immune regulation and phagocytic ability, promoting the invasion, proliferation, adhesion, etc. of ectopic cells. Regulating their polarization can affect the course of thin endometriosis, but the relevant mechanism is still inconclusive. Therefore, discovering and exploring the functions and molecular mechanisms of peritoneal macrophages associated with thin endometrial disease, and targeting reprogrammed macrophages to have positive immune function to clear lesions or delay the course of the disease, is a clinical challenge that needs to be overcome. The relationship between endometriosis and peritoneal macrophages is shown in Figure 1.

Macrophages are important regulatory cells for innate and adaptive immunity in response to inflammation, injury, repair, and fibrosis in diseases. The recent consensus is that the occurrence and development of thin endometrial disease are closely related to the process of local pelvic inflammatory disease, and immune cell function in the abdominal environment of patients with thin endometrial disease is abnormal. Peritoneal macrophages are an important component of the abdominal immune microenvironment and play a crucial role in many diseases [10]. Ovarian cancer peritoneal macrophages can help tumor cells escape immune surveillance and promote tumor cell proliferation. The occurrence of thin endometrium is related to the number and function of peritoneal macrophages, but there are conflicting regulatory mechanisms. In thin endometriosis, the phagocytic activity of peritoneal macrophages towards ectopic endometrial cells is reduced, which facilitates the colonization and growth of ectopic lesions, indicating the presence of immune dysfunction. Macrophages

play an important role in the formation and control of chronic inflammatory responses around the pelvic and abdominal cavity, which occurs in all stages of thin endometrial disease. In the early stages of thin endometrial disease, macrophages recruited from the lesion cells, fragments, and other stimuli can control the inflammatory response, engulf thin endometrial cells, and promote post inflammatory repair. As the disease progresses, early M1 macrophages are exhausted, and the immune microenvironment promotes macrophage polarization imbalance. M2 macrophages gradually become dominant, allowing thin endometrial cells to evade immunity and promote disease progression to varying degrees.

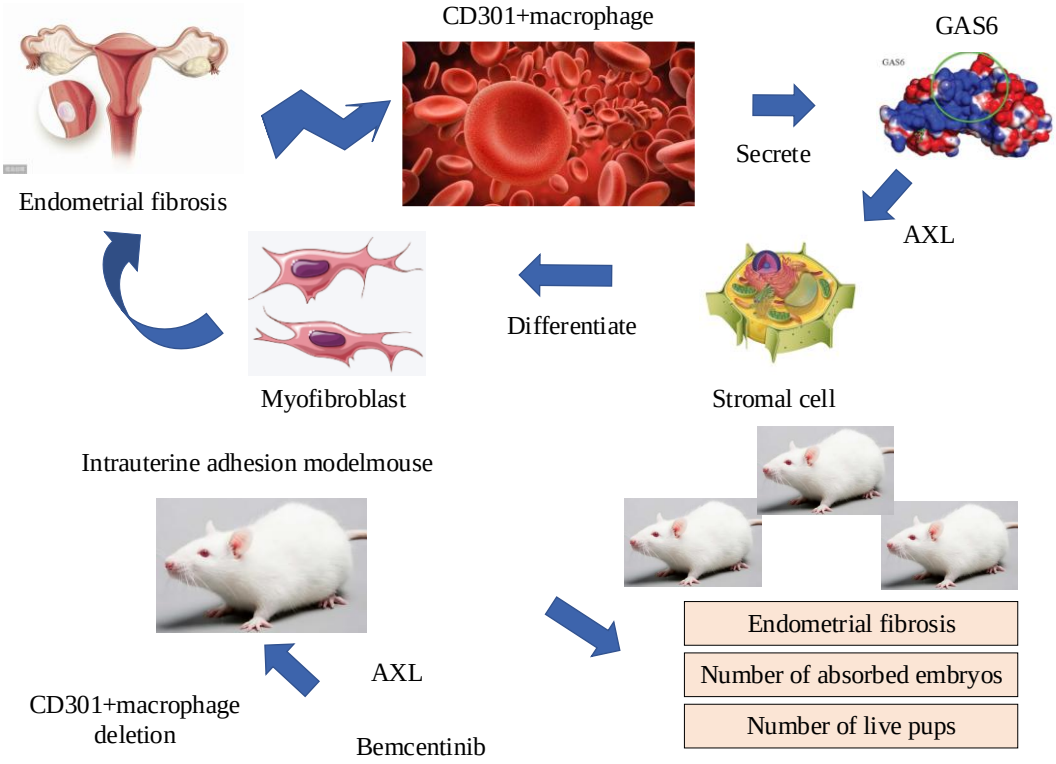

Figure 1: Endometriosis and peritoneal macrophages

3.2 Relationship between T lymphocytes and thin endometrium

In the endometrium, you will find that two out of every three T cells are CD8+, and this balance remains stable even during ovulation or pregnancy in women [11]. The scientific community's understanding of the work done by these endometrial T cells is still quite fragmented, with most research focused on the decidual tissue. T cells are like small factories that produce cytokines, and the Th2 team is the key to managing so-called humoral immunity. This involves cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13. On the other hand, Th1 cells play a leading role in cellular immunity, utilizing cytokines such as IL-2, IFN - γ, and TNF - β to accomplish this task. People have noticed that in the thin endometrium, genes driving oxidative phosphorylation processes are inhibited, and natural killer cell and T cell receptor signaling channels are enhanced. These adjustments signify a significant improvement in the immune system's ability to respond to specific threats. In addition, patients with persistent miscarriage often have a higher proportion of pro-inflammatory cytokines and anti-inflammatory cytokines in their blood. The imbalance of IFN - γ/IL-4 and IFN - γ/IL-10 may be just one of the multiple ways in which the endometrium of women becomes thinner. According to logistic regression, the probability equation for joint diagnosis prediction is as follows:

$$f = -0.273 \times \mathrm{CD}^{+} + 0.218 \times \mathrm{CD}^{+} + 2.616 \times TNF - \alpha + 1.617 \quad (1)$$

ROC curves for diagnosing thin endometrium by plotting CD4+, CD8+, TNF - α in the blood alone and in combination with three indicators [12]. The area under the curve (AUC) of the joint diagnosis is the largest, at 0.934, with sensitivity and specificity of 91.7% and 83.3%, respectively (P<0.05). See Table 1.

T lymphocytes are a group of cells that can regulate the immune function of the body. CD4+T cells represent a key subset of helper lymphocytes, while CD8+T cells constitute an inhibitory lymphocyte population with cytotoxic properties. These two cell types are typically in a specific equilibrium state, and the ratio between CD4+and CD8+T cells is an intrinsic biomarker reflecting the body's immune function status. The results of this study indicate a significant correlation between the levels of CD4+T cells, CD8+T cells, and CD4+/CD8+ratio and the pathogenesis of thin endometrium. CD4+is an independent protective factor for thin endometrium, while CD8+is an independent risk factor. The proportion of CD4+lymphocytes and CD4+/CD8+ratio in peripheral blood of patients with thin endometrium were significantly lower than those in the control group, while the proportion of CD8+lymphocytes was significantly increased compared to the control group, which is consistent with recent research reports by Li Hong. It is speculated that the thin endometrium may be due to an imbalance in the body's immune system, which disrupts the normal proportion of immune cells and disrupts their activation and inhibition functions, leading to pathological immune responses and changes in tissue cells.

Table 1: Value analysis of CD4+, CD8+, and TNF-α alone and in combination for the diagnosis of thin endometrium

Index	AUC	SE	P	95% CI	Sensitivity (%)	Specificity (%)	Best cutoff value [(%), pg/mL]
CD4+	0.857	0. 030	<0.001	0.799~0.915	61. 1	95.8	40. 00
CD8+	0.813	0. 037	<0.001	0.741~0.886	87. 5	66. 7	27. 40
TNF-α	0. 875	0. 028	<0.001	0.820~0.930	97. 2	63. 9	1. 22
Combined diagnosis	0. 934	0. 020	<0.001	0.896~0.972	91.7	83.3	--

3.3 Relationship between NK cells and thin endometrium

NK cells are pivotal in the body's cellular immune defense due to their ability to destroy cells. When these cells become hyperactive in targeting and eliminating endometrial cells, it might lead to a thin endometrium. Studies have shown that the NK cells found in the endometrium have a distinct profile compared to those in the bloodstream and macrophages [13]. During the phase of tissue growth, there's a relatively low count of NK cells in the endometrium, but this number climbs as the menstrual cycle draws near. Understanding the hyperactivation of NK cells might pave the way for treating thin endometria, with treatments potentially including slowing down cell aging, clearing collagen from the stroma, and quelling an overly aggressive immune response. Misfiring of immune cells and their dysfunctional interactions with other cell types might just be the root cause behind the thinning of the endometrium. The expression of NKG2D molecules in endometrial (A), thin endometrial (B), and normal endometrial (c) tissues after immunohistochemical staining is shown in Figure 2.

Immunohistochemical staining revealed that the expression intensity of NKG2D varies in different tissues (Figure 2). Quantitative analysis revealed that the expression level of NKG2D in patients with thin endometrium was lower than that in healthy women (A vs C, both P<0.01);

The expression level of NKG2D in thin endometrial tissue was also lower than that in its in-situ endometrium (A vs B, both P<0.01); There was no significant difference in the expression level of NKG2D between the endometrium of patients with thin endometrium and the endometrium tissue of healthy women (both P>0.05).

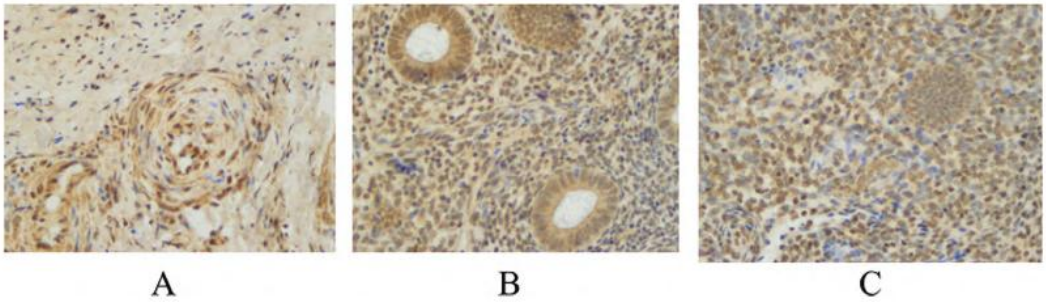

Figure 2: Expression of NKG2D molecules in different endometrial tissues after immunohistochemical staining (× 400)

At present, research on NK cells in patients with thin endometrium is mostly limited to peripheral blood and peritoneal fluid levels [14]. Some studies have shown that the percentage of CD56+and/or CD16+NK cells in peripheral blood and peritoneal fluid of patients with thin endometrium is not significantly abnormal. Only a few studies suggest a decrease in the percentage of CD16+CD57+or CD16+CD56+NK cells, and in some cases, an increase in the percentage of CD56+or CD16+CD56+NK cells. Research by Drury et al. has shown that the percentage of NK cells in the endometrial tissue of both thin and non-thin endometrial patients gradually increases from the proliferative phase and reaches its highest level in the late secretory phase. However, throughout the menstrual cycle, the percentage of NK cells in ectopic lesions is significantly lower. Our study also confirmed that there is a decrease in the number of NK cells in the thin endometrium of patients with ovarian type thin endometrium, while no similar phenomenon was observed in the endometrial tissue of patients with ovarian type thin endometrium. Therefore, we believe that the decrease in the number of NK cells in the tissues of patients with thin endometrium may promote the occurrence and development of EM. At the same time, the in-situ endometrium of patients with thin endometrium does not induce the occurrence and development of related diseases through the NK cell pathway. In addition, there were no significant differences in age, body mass, and surgical timing among the patients in this study, which has avoided the influence of menstrual cycle on NK cell levels.

4 Relationship between cytokines and thin endometrium

4.1 Relationship between G-CSF and thin endometrium

The reproductive system, particularly the endometrium, produces G-CSF, whose secretion is modulated by sex hormones [15]. This cytokine plays a vital role in endometrial repair and blood vessel formation. Through its dual action of stimulating vascular growth and preventing programmed cell death, G-CSF facilitates tissue regeneration while minimizing cellular damage. Beyond its regenerative properties, G-CSF modulates immune responses by expanding regulatory T cell populations, activating dendritic cells, suppressing natural killer cell toxicity, and biasing the immune response toward a Th2-dominant profile. Research by F. Davari-Tanha et al. demonstrates G-CSF's significant involvement in endometrial phagocytic activity and oxidative metabolism, along with its regulation of implantation-critical genes associated with

vascular adaptation, immune modulation, and cellular adhesion. Notably, insufficient G-CSF concentrations can disrupt immune homeostasis, potentially impairing endometrial growth and contributing to endometrial thinning. Perform statistical analysis using SPSS 26.0. The data is represented as:

$$y = \overline{x} \pm s \tag{2}$$

One way ANOVA is used to compare differences between three or more groups, while within group comparisons are performed using graph-based tests. $P<0.05$ indicates a statistically significant difference, and all experiments should be repeated at least 3 times. To determine the regulatory role of G-CSF in fibrosis, pcDNA3.1-G-CSF and si-G-CSF were transfected into cells to overexpress and silence G-CSF. The qRT PCR assay results showed that the expression of G-CSF was upregulated after transfection with pcDNA 3.1-G-CSF, while the expression of G-CSF was downregulated after transfection with si-G-CSF. See Figure 3 for details [16].

The above results demonstrate that pcDNA 3.1-G-CSF and si-G-CSF have high overexpression efficiency and knockdown efficiency, respectively, in HESC cells. G-CSF is an important molecule of the colony-stimulating factor family, which can promote the release, differentiation, and proliferation of neutrophil lines. In the field of reproduction, G-CSF is closely related to processes such as follicular development, endometrial shedding, and proliferation. In 2011, Cleicher et al. reported that G-CSF can regulate the microenvironment of endometrial cells and promote endometrial growth. G-CSF intrauterine infusion can increase endometrial thickness and improve clinical outcomes in in vitro fertilization embryo transfer patients. However, the specific mechanisms and regulatory factors by which G-CSF increases endometrial thickness and improves the uterine microenvironment are still unclear. The differential expression pattern of G-CSF in H8 cells was revealed using qRT PCR technology. It was found that overexpression of G-CSF may inhibit the expression of N-cadherin and Vimentin, promote the expression of E-cadherin and Zo -, while silencing G-CSF may promote the expression of N-cadherin and Vimentin, inhibit the expression of E-cadherin and Z0-1. These data demonstrate that G-CSF may be involved in the EMT process of H8 cells.

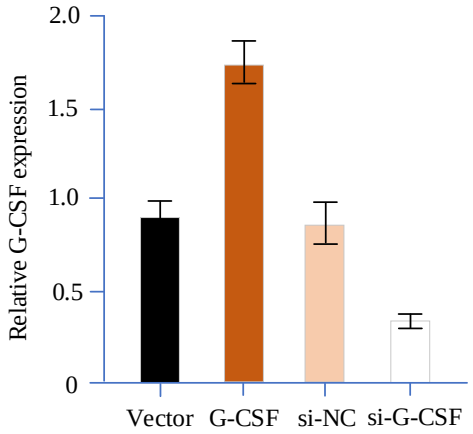

Figure 3: Expression of target genes in G-CSF overexpressing and knocking down cell lines

4.2 Relationship between vascular endothelial growth factor and thin endometrium

Vascular endothelial growth factor (VEGF) is a versatile signaling protein that plays a key role

in regulating blood vessel formation by selectively acting on vascular endothelial cells. By binding tightly to its receptors on these cells, VEGF stimulates their division and facilitates the development of new capillaries [17].

During the menstrual cycle, studies show that thin endometrial tissue exhibits higher blood flow resistance and elevated uterine artery resistance indices compared to healthy endometrium. This impaired vascular development coincides with stunted glandular epithelial growth, fewer blood vessels, and reduced expression of the endothelial marker CD38. The deficiency in VEGF likely disrupts proper blood vessel formation and maturation, leaving the endometrium too underdeveloped to support embryo implantation.

Interestingly, the relationship between blood flow and endometrial thickness appears cyclical. Increased vascular resistance may hinder glandular epithelial growth, thereby lowering endometrial VEGF levels. This VEGF shortage then further compromises blood vessel integrity, diminishing endometrial perfusion—a self-perpetuating cycle that ultimately weakens the uterus's ability to receive an embryo [18].

Using Haploview software, Hardy Weinberg equilibrium tests were performed on the genotype distribution of the -2578C/A, -1154G/A, -460C/T, and+936C/T polymorphism sites in the promoter region of the VECF gene. The odds ratio (OR) and 95% confidence interval (CI) representing relative risk were calculated using unconditional logistic regression, with P<0.05 indicating statistically significant differences. The calculation formula for unconditional logistic regression is as follows:

$$p = \frac{1}{1+e^{-\beta_0-\beta_1 X_1-\beta_2 X_2-\cdots-\beta_n X_n}} \tag{3}$$

where, p represents the probability of an event occurring; $\beta_1,\beta_2,\cdots,\beta_n$ is the coefficient of the independent variable; β_0 is the intercept term; $X_1,X_2,\cdots,X_n$ is the independent variable value.

This formula maps the linear regression results to the probability interval [0,1] using the Sigmoid function. When the predicted probability p exceeds 0.5, the event is judged to have occurred (i.e., the probability is greater than 0.5).

The VEGF gene interacts with the expression of multiple susceptible genes in thin endometrium, and the results of the unconditional logistic regression are shown in Figure 4.

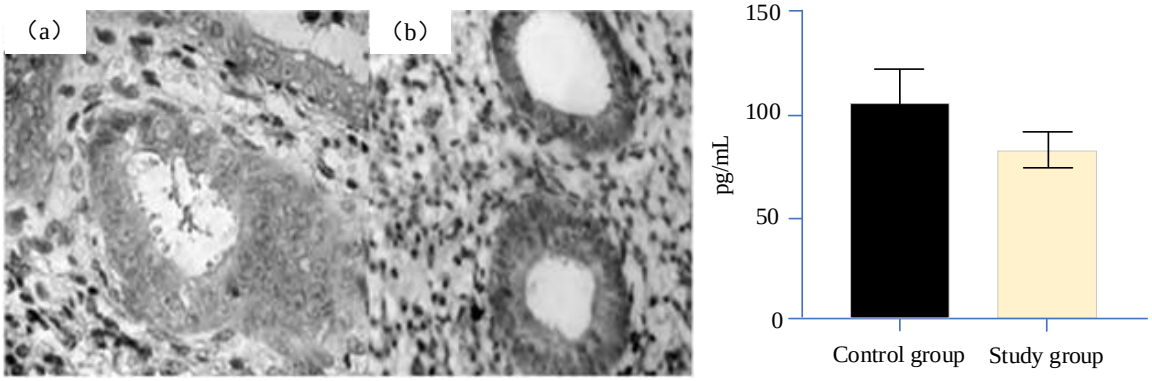

Figure 4: Comparison of serum VECF levels and endometrial VECF expression between two groups

Research has found that the -2578C/A polymorphism site in the promoter region of the VECF gene is not associated with thin endometrium [19]. It is speculated that this site does not alter the transcriptional activity of VECF and affect endometrial angiogenesis, which is consistent with previous studies suggesting that the -2578C/A polymorphism site in the

promoter region of the VECF gene cannot effectively regulate VECF activity. At present, there is still no clear functional study on the polymorphism site of VECF gene promoter region -1154C/A, but research has found that the -1154G/A polymorphism site is associated with high expression of VEGF. This study found that serum VECF levels were reduced in patients with thin endometrium, and the polymorphism site of VECF gene promoter region-1154C/A was not associated with the occurrence of this disease, indirectly confirming the role of VECF gene promoter region-1154G/A in enhancing VECF activity, but not in reducing VECF activity.

4.3 Relationship between leukemia inhibitory factor and thin endometrium

Leukemia inhibitory factor (LIF) seems to play an important role in the development of blood vessels in the endometrium and placenta, particularly by regulating the levels of vascular endothelial growth factor (VEGF) in the endometrium [20]. Research suggests that LIF may become a biomarker for measuring endometrial preparation in women with repeated implantation failures. This means that insufficient LIF production may hinder the formation of new blood vessels in the endometrium, ultimately leading to implantation issues. RT real-time PCR experiments showed that the mRNA levels of LIF in two endometrial cancer cells (HEC-1B, RL95-2) overexpressing the model significantly increased ($P<0.05$, Figure 5A). ELISA experiments showed that the two endometrial cancer cells overexpressing the model were able to secrete more LIF within 24 hours ($P<0.05$, Figure 5B).

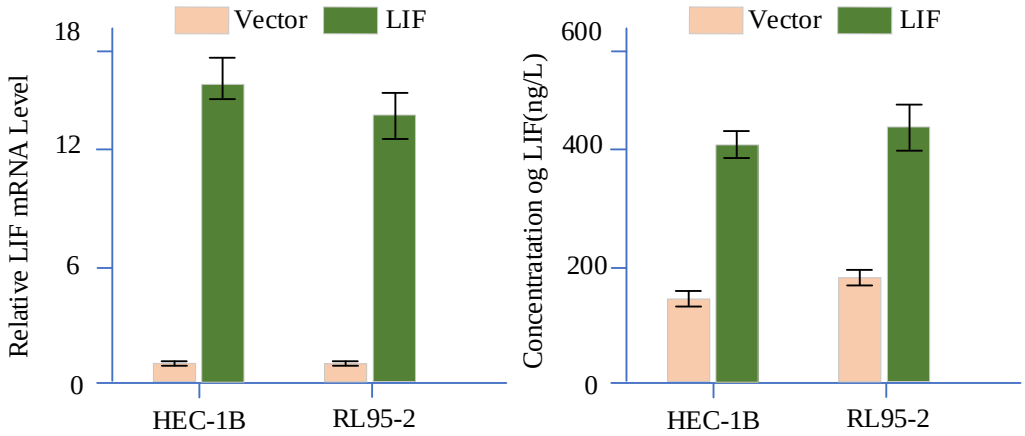

Figure 5: Establishment of Overexpressed LIF Endometrial Cancer Cells

The receptor of LIF is leukemia inhibitory factor receptor (LIFR). Shi et al. pointed out that on the animal model of pancreatic cancer, neutralizing LF in the environment or knocking out LIFR of cancer cells can significantly inhibit the malignant progression of pancreatic cancer, enhance the effectiveness of chemotherapy drugs, and prolong the survival time of animals; The mechanism may be related to LIF's ability to enhance the phosphorylation and transcription levels of STAT3 through LIFR, thereby regulating gene expression related to disease progression and chemotherapy resistance. The research results of this article also show that even though cisplatin and paclitaxel have different anti-cancer mechanisms, they can both be observed to damage mitochondrial function and induce cell apoptosis. Overexpression of LIF can activate the STAT3 signaling pathway, upregulate anti apoptotic proteins Bcl-2 and Bcl xL, downregulate inducible apoptotic proteins Bad and Bax, stabilize mitochondrial membrane potential, and reduce cell apoptosis; Based on the above studies 21-23, it can be clarified that L-positive F is involved in the malignant progression and chemotherapy resistance of various

tumors, and is a potential therapeutic target [21]. It is worth noting that normal endometrial glandular epithelium synthesizes and secretes L-positive cells in a menstrual cycle dependent manner, which is crucial for initiating blastocyst implantation and maintaining early embryonic development. LIF expression is upregulated in proliferative endometrium and endometrial cancer, and may be involved in the malignant progression of endometrial cancer. Therefore, further exploration of the role of LIF in the female reproductive system and embryo formation and development may avoid potential risks associated with anti LIF therapy.

5 Immunomodulatory treatment of thin endometrium

5.1 Traditional treatment

5.1.1 Estrogen

Estrogen therapy plays a crucial role in preventing the reformation of adhesions and encouraging the regrowth of the endometrial lining, which results in its growth and thickening. Moreover, estrogen suppresses the rise of serum TGF-β1, epidermal growth factor, and PDGF-BB levels, thereby boosting the concentrations of ESR1, MMP-9, EGF, and IGF-1. As a hormone-sensitive tissue, the endometrium responds to estrogen by proliferating, engaging estrogen receptors, and facilitating the rebuilding of its vascular network to repair injured regions [22].

5.1.2 Growth hormone

Research suggests that growth hormone (GH) supplementation can significantly improve endometrial thickness and boost implantation success rates [23]. When used as part of a combined treatment approach, GH therapy appears to enhance uterine lining development in women struggling with infertility while also optimizing the endometrium's receptivity. These benefits are thought to occur through GH's ability to stimulate endometrial blood flow, thereby activating key genes and proteins involved in creating a more favorable uterine environment for implantation.

5.1.3 Pelvic neuromuscular electrical stimulation

Recent studies have shown that acupuncture and moxibustion can significantly improve endometrial receptivity by enhancing integrin α v β 3, HOXA10, HBEGF, estrogen and progesterone receptors and other key biomarkers. In addition, electroacupuncture has been proven to optimize uterine microcirculation, upregulate hormone receptor expression, increase serum estrogen levels, promote endometrial regeneration, thereby increasing clinical pregnancy rates and live birth rates, while alleviating pain and anxiety during embryo transfer. Recent research findings also suggest that electroacupuncture may stimulate the proliferation and migration of stem cells, thereby promoting tissue regeneration at the site of injury [24].

For women with thin endometrium and infertility problems, pelvic neuromuscular electrical stimulation (NMS) has shown considerable application prospects. Related studies have shown that NMS can not only promote an increase in endometrial thickness, but also improve blood flow perfusion, and maintain luteinizing hormone levels without interference before ovulation. The results of these observations indicate that NMS can be used as a viable treatment option by enhancing blood circulation in the pelvic area, reducing vascular resistance, and improving blood supply to the endometrium and uterine muscles. By regulating the activity status of

vascular smooth muscle, this intervention measure can effectively promote the growth and development of the endometrium.

5.2 Regenerative medicine

5.2.1 Platelet-rich plasma

Platelet rich plasma (PRP) is a concentrated solution extracted from the patient's own blood. Due to its regenerative properties, it has been widely used in dermatology, orthopedics, and cosmetic surgery [25]. However, its application in reproductive medicine remains a controversial topic. PRP is rich in cytokines and growth factors stored in platelet alpha granules, showing the potential to stimulate tissue repair at the cellular level. Emerging research suggests that it may also promote endometrial proliferation.

When introduced into injured or inflamed tissue, activated platelets release a mixture of growth factors and cytokines from their alpha granules. These bioactive molecules play a key role in fibroblast activation, immune cell recruitment, and regulation of smooth muscle and mesenchymal stem cell activity, which are critical processes for tissue repair and angiogenesis. Although some clinical studies have reported improvements in endometrial growth and pregnancy rates after infusion of PRP, its efficacy in promoting endometrial thickening remains controversial.

Animal studies have revealed the mechanism of PRP, indicating that it enhances endometrial cell proliferation and migration while reducing fibrosis. It also regulates gene expression, upregulates TGF - α and EGFR, both of which are crucial for tissue regeneration. PRP appears to inhibit harmful cell death pathways such as ferroptosis, pyroptosis, and excessive autophagy, while restoring reduced levels of TGF - α. It is worth noting that EGFR is a receptor associated with cell growth and movement, and its expression increases under the action of PRP.

In addition to these effects, PRP also stimulates endometrial mesenchymal stem cells (EnMSCs), promoting their migration, proliferation, and differentiation into functional endometrial cells. This process may have the potential to restore endometrial function in conditions such as thin endometrium (TE).

5.2.2 Growth factors

For a long time, growth factors have been considered as key cellular messengers that promote tissue regeneration, accelerate wound repair, and drive cell proliferation. New evidence suggests that these biochemical signals may also directly stimulate the dilation of endometrial tissue.

5.2.3 Mesenchymal stem cells

Stem cells have the remarkable capacity to differentiate directly into specialized cells, facilitating tissue repair and delivering therapeutic benefits. Their dual capabilities—self-renewal and multipotency—allow them to transform into various cell types. Beyond regeneration, these versatile cells modulate immune responses by releasing cytokines and chemokines, influencing B cells, NK cells, and macrophages, which is pivotal in endometrial repair [26].

Broadly classified by origin, stem cells fall into two categories: embryonic and adult stem cells. Mesenchymal stem cells (MSCs), the most extensively studied type, have been successfully harvested from diverse sources, including bone marrow, adipose tissue, umbilical cord blood, menstrual blood, and amniotic fluid, with promising applications in clinical trials.

Research demonstrates that exosomes from human umbilical cord mesenchymal stem cells (Huc-MSCs) significantly aid endometrial regeneration in rat models with immune-mediated damage. These exosomes not only boost CD163+ M2 macrophage polarization but also mitigate inflammation via miRNA-driven immune modulation.

MSCs enhance endometrial thickness and fine-tune immune responses through their distinctive homing and immunomodulatory properties. By tempering lymphocyte activity, they achieve both anti-inflammatory and immune-balancing effects. Furthermore, MSCs exert control over T-cell function and proliferation, harmonizing Th1/Th2 dynamics while amplifying regulatory T-cell (Treg) activity. They also suppress B-cell and NK-cell proliferation and block dendritic cell maturation, reinforcing their therapeutic potential.

5.2.4 Human umbilical cord mesenchymal stem cells

In their research, Ying Zhong and colleagues revealed that when human umbilical cord mesenchymal stem cells (Huc-MSCs) are encased within PF-127, a heat-sensitive, biodegradable hydrogel cleared by the FDA, they're able to stimulate the release of growth factors in response to elevated levels of IL-1β in the vicinity. This triggers the development of fresh vascular networks. The IL-1β-rich environment at the site spurs HUC-MSCs to secrete factors crucial for the growth of endometrial tissue, which in turn repairs thin endometria, bolstering gland density and thickness. While the exact mechanisms of this process in regenerating thin endometria are still up in the air, the study did find that when HUC-MSCs are triggered by IL-1β, there's a notable uptick in the expression of angiogenic markers, like bFGF, EGF, and HGF. This boost in bFGF, which is collagen-bound, is instrumental in promoting angiogenesis and healing damaged uteri. PF-127 also manages to keep the Huc-MSCs lingering longer, which improves their ability to rejuvenate endometrial tissue. Wrapping hUC-MSCs in a temperature-sensitive hydrogel has the potential to not only thicken the endometrium but also boost vascular formation. Yet, variations in the stem cells obtained from different umbilical cord donors can lead to inconsistent results in treatment efficacy [27].

5.3 G-CSF, granulocyte colony-stimulating factor

In the real world of clinical work, G-CSF is usually given via intrauterine infusions instead of intravenous shots to treat thin endometria. This therapy has the potential to beef up the endometrium's thickness and ability to accept implantation by a multi-faceted approach. It enhances the production of VEGF, a key player in getting blood vessels growing and circulation flowing; boosts the count of those regulatory T cells; wakes up the dendritic cells; quiets down the attack mode of the NK cells; and ups the ante on the leukocyte inhibitory factor (LIF). Together, these actions help with follicle growth, egg release, and embryo attachment, which is a win for treating failed implantations time and time again. Despite the lack of conclusive evidence on how G-CSF works on thin endometria, it's thought that it might trigger some immune responses that mend the damaged gland and tissue areas. Research suggests that G-CSF can get in on the act of trophoblast penetration by increasing levels of MMP-2 and VEGF, which is a big deal for boosting endometrial acceptance [28]. Plus, it seems G-CSF can help with regenerating the endometrium by getting stem cells from the bone marrow into the bloodstream, where they transform into the right types of cells [29, 30].

6 Conclusion

Main work: This article provides a review of the research progress in the field of endometrial thin immunity. Firstly, it is introduced that there is currently no unified definition of endometrial

thinness, and its etiology is unknown and existing treatment methods are limited. However, emerging research suggests that immune pathways play a key role in successful embryo implantation, and immune response dysregulation may be associated with endometrial thinness. Then, the relationship between immune cells and endometrial thin cells was elaborated in detail, including the different effects of macrophages on the proliferation or apoptosis of endometrial thin cells under different polarization states, as well as their role in promoting disease progression by regulating inflammatory response and immune microenvironment at different stages of the disease; The CD4+, CD8+, and CD4+/CD8+ratios in T lymphocytes are associated with the development of endometrial thinning, and an imbalance in their ratios may trigger pathological immune responses; The changes in the quantity and activity of NK cells in the endometrium may be related to the thinness of the endometrium, and their excessive activation may damage endometrial cells. At the same time, the relationship between cytokines and endometrial thinning was explored, such as the important role of G-CSF in endometrial repair, angiogenesis, and immune regulation. Insufficient concentration of G-CSF may affect endometrial growth; VEGF is crucial for angiogenesis, and a lack of VEGF in the thin tissue of the endometrium can hinder vascular development, leading to insufficient endometrial perfusion; LIF is involved in the development of endometrial and placental blood vessels, and its abnormal expression may affect endometrial receptivity. In addition, immunomodulatory therapy for endometrial thinning was introduced, including traditional treatments such as estrogen, growth hormone, pelvic neuromuscular electrical stimulation, as well as treatment methods in the field of regenerative medicine such as platelet rich plasma, growth factors, mesenchymal stem cells, human umbilical cord mesenchymal stem cells, and G-CSF.

Future research direction: Although some progress has been made in current research, there are still many unknown areas in the thin immune mechanism of the endometrium. Future research needs to further explore the specific mechanisms of immune cells and cytokines in the occurrence and development of endometrial thinning, and clarify the interaction network between various factors. At the same time, more large-scale, multicenter clinical trials are needed to validate the efficacy and safety of existing immunomodulatory therapy methods and optimize treatment plans. In addition, efforts should be made to search for new immune related biomarkers to provide more accurate basis for early diagnosis, disease monitoring, and prognosis evaluation of thin endometrium, thereby improving the fertility outcomes of patients.

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