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Effects of Intrauterine Infusion of G-CSF and HCG on Peripheral Blood Treg and Pregnancy Outcome in Patients with Thin Endometrium Undergoing Frozen-thawed Embryo Transfer

Hui-Jun Yu[1], Qi Wan[1], Li Tan[1], Xing-Yu Lv[1*]

[1]Chengdu Xinan Gynecology Hospital, Chengdu, 610051, China

ABSTRACT

Background: Patients with thin endometrium undergoing frozen-thawed embryo transfer often encounter challenges with pregnancy outcomes. Enhancing endometrial receptivity and immune tolerance may improve these outcomes.

Objective: To investigate the effects of intrauterine perfusion of granulocyte colony-stimulating factor (G-CSF) and human chorionic gonadotropin (HCG) on regulatory T cells (Tregs) and pregnancy outcomes in patients with thin endometrium undergoing frozen-thawed embryo transfer.

Methods: 150 patients with thin endometrium were randomly assigned to three groups: a control group that received no intervention, an HCG group, and a G-CSF group. The effectiveness of the treatments was assessed by comparing uterine parameters, Treg levels, and pregnancy outcomes across the groups.

Results: The HCG and G-CSF groups exhibited significant improvements compared to the control group, including increased endometrial thickness, enhanced blood flow, higher expression of endometrial receptivity markers (integrin αvβ3, osteopontin), and elevated Treg levels. Notably, the G-CSF group demonstrated even greater enhancements compared to the HCG group, with significantly higher endometrial thickness, better blood flow, increased receptivity markers, and elevated Treg levels. Additionally, the G-CSF group achieved significantly higher biochemical and clinical pregnancy rates compared to both the HCG and control groups. This highlights the potential of G-CSF in improving pregnancy outcomes for patients with a thin endometrium.

Conclusion: The intrauterine perfusion of G-CSF significantly enhanced pregnancy outcomes in patients with thin endometrium by improving endometrial blood flow, immune tolerance, thickness, Treg induction, and embryo implantation. These findings suggest that G-CSF could be a promising therapeutic option for this patient population.

Keywords: Chorionic gonadotropin, Embryo transfer, Endometrium, Granulocyte colony-stimulating factor, T-Lymphocytes

*Corresponding author:
Xing-Yu Lv,
No.66, No.88, Bisheng Road, Jinjiang District, Chengdu City, Sichuan Province, 610051, China
Email: lxyy323@163.com

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INTRODUCTION

Infertility in women of childbearing age refers to couples who intend to have children, have been living together for 1 year or more without using contraception, and have not achieved a pregnancy. Epidemiological data indicate that the worldwide prevalence of infertility is as high as 15%-20%, making it the third most common disease after cancer and cardiovascular disease (1). It has been reported that the incidence of infertility is affected by body mass index (BMI), abortion, the abuse of emergency contraception, sleep, work pressure, ionizing radiation, and other factors (2). Currently, the incidence of infertility is increasing year by year, and it significantly affects the quality of life of patients. Assisted reproductive technology is an important means to address infertility. Since the first case of successful freeze-thaw embryo transfer resulting in clinical pregnancy in 1983, this technique has become an essential component of assisted reproductive technology (3).

It has been reported that the thinner the endometrium, the lower the success rate of transplantation (4). However, traditional estrogen and gonadotropin-releasing hormone agonists are often ineffective in increasing endometrial thickness. Therefore it is particularly important to find effective methods to increase endometrial thickness. Intrauterine perfusion is an important method to increase the success rate of embryo transfer by introducing substances that can increase uterine endometrial thickness and improve endometrial receptivity. Moreover, an increasing amount of evidence has shown that local immune cells and immune tolerance at the implantation site play a positive role in embryo implantation (5). Human chorionic gonadotropin (HCG) is a glycoprotein hormone that has a similar effect to follicle-stimulating hormone. It can promote the secretion of estradiol by endometrial epithelial cells, and change the uterine environment and immune tolerance. Intrauterine perfusion of HCG can increase patients' endometrial thickness and provide favorable support for embryo colonization (6). However, despite the positive effects of HCG on the uterine environment, clinics have found that the clinical pregnancy rate remains low (7).

Granulocyte colony-stimulating factor (G-CSF), as a glycoprotein that can promote angiogenesis and immune regulation, promotes the proliferation and activation of neutrophils in the thin endometrium and can affect the function of endometrial cells in the female reproductive system (8). G-CSF is well known for its ability to enhance the proliferation and activation of neutrophils. This cytokine binds to the G-CSF receptor on the surface of neutrophil precursors in the bone marrow, stimulating their differentiation and proliferation. Additionally, G-CSF enhances the functional activity of mature neutrophils by increasing their phagocytic ability and production of reactive oxygen species, which are critical for pathogen clearance (9). While the primary role of neutrophils is to defend against infection, their function in the reproductive system, particularly in the endometrium, involves remodeling tissue and influencing immune tolerance. In the context of a thin endometrium, G-CSF's effect on neutrophils may extend beyond traditional immune responses. The activation and recruitment of neutrophils to the endometrium could facilitate local immune modulation and tissue remodeling, enhancing the endometrial environment for embryo implantation. This is supported by studies indicating that neutrophils and other immune cells contribute to the preparation of the endometrium for implantation by modulating local inflammation and promoting angiogenesis (10). Exogenous administration of G-CSF can modify the expression of endometrium-related genes and enhance the implantation rate of embryos in the endometrium (11). Maximizing the success rate of embryo transfer is crucial for patients with thin endometrial. Therefore,

we analyzed and compared the effects of intrauterine perfusion of G-CSF and HCG on Treg expression and pregnancy outcomes in thin endometrium patients who underwent freeze-thaw embryo transfer.

SUBJECT AND METHODS

Subjects

This study was approved by the Ethics Committee of Chengdu Xinan Gynecology Hospital (No. K2021025). All patients signed informed consent. A total of 150 patients with thin endometrium who underwent freeze-thaw embryo transfer at our hospital from June 2021 to June 2022 were included in this study. Patients in the control group were aged 24 to 36 years old with an infertile period of 3 to 11 years. In the HCG group, patients were aged 24 to 35 years old with an infertile period of 3 to 10 years, and in the G-CSF group, patients were aged 25 to 35 years old with an infertile period of 3 to 12 years. The general clinical data for the three groups are shown in Table 1.

Inclusion criteria: (1) voluntary acceptance of embryo transfer; (2) individuals planning to undergo freeze-thaw embryo transfer in this cycle, whit endometrial thickness of 7mm or less during the previous cycle of fresh embryo suppression or natural cycle of freeze-thaw embryo transfer, and no pregnancy after transfer; (3) Under the age of 40; (4) having 1 or more high-quality frozen embryos; and (5) normal range of basic endocrine hormone levels; (6) Embryo transfer to be conducted on the fifth day of menstruation during the frozen-thawed embryo transfer cycle.

Exclusion criteria: (1) chromosomal abnormalities in either spouse; (2) uterine abnormalities, such as uterine fibroids and other organic diseases; (3) diabetes, thyroid abnormalities, and other medical conditions; (4) patients positive for autoimmune antibodies; (5) luteal insufficiency and hyperprolactinemia; (6) dysfunction of the heart, liver, or kidneys; and (7) mental abnormalities. They were randomly divided into three groups using the number table method, including the control group (no intervention), the HCG group (intrauterine perfusion of HCG), and the G-CSF group (intrauterine perfusion of G-CSF), with 50 cases in each group.

Endometrial Preparation for Hormone Replacement Cycles

All patients in the three groups underwent endometrial preparation for the hormone replacement cycle and were instructed to take oral estradiol valerate at a dose of 4- 6 mg per day (12) starting on the third day of their menstrual cycle. A transvaginal ultrasound examination and serum examination were performed one week later to measure the endometrial thickness and the level of estradiol valerate. The dosage of estradiol valerate was adjusted based on the measurement results. Estradiol valerate was used for

Table 1. Comparison of three groups of general Information ($\bar{x} \pm s$)

group	Average age (years)	Average years of infertility (years)	Mean body mass index (kg/m^2)	Base estradiol (pg/ml)	Basal follicle stimulating hormone (mIU/ml)	Basal luteinizing hormone (mIU/ml)
Blank group (n=50)	29.11 + / - 2.27	6.65 + / - 1.28	21.88 + / - 1.29	43.46 + / - 4.11	7.19 + / - 1.14	4.22 + / - 0.26
HCG group (n=50)	29.23 + / - 2.24	6.73 + / - 1.24	21.80 + / - 1.38	43.70 + / - 4.25	6.99 + / - 1.05	4.27 + / - 0.30
G-CSF group (n=50)	29.13 + / - 2.43	6.78 + / - 1.43	21.98 + / - 1.43	43.52 + / - 4.25	7.12 + / - 1.08	4.24 + / - 0.29
F	0.266	0.479	0.300	0.287	0.913	0.891
P	0.791	0.633	0.765	0.775	0.364	0.375

14 days when the estradiol level was>100 pg/ml and endometrial thickness was ≥8 mm. Progesterone (progesterone injection 80mg/day) was then added to transition the endometrium from the proliferative stage to the secretory stage. The day when progesterone was added was referred to as progesterone conversion day, and the frozen-thawed embryo transfer took place four days later. Another physician reviewed the endometrium to confirm its accuracy.

Intrauterine Perfusion of HCG

Patients in the HCG group underwent intrauterine infusion of HCG on the day of progesterone conversion. After routine vulvovaginal disinfection, 0.5 ml of normal saline was drawn into a syringe to dissolve 250 μg of HCG (13). The HCG was then slowly injected into the uterine cavity 2 cm away from the uterine floor. After receiving the injection, the patients were positioned supine at hip height on the examination bed for 15-20 min. Another physician reviewed the results of the endometrium and hormone infusion to confirm their accuracy.

Intrauterine Infusion of G-CSF

Patients in the G-CSF group recieved intrauterine infusion of G-CSF on the day of progesterone conversion. After routine disinfection of the vulva and vagina, 150 μg of G-CSF (14) was drawn into a syringe, and slowly injected into the uterine cavity under ultrasound guidance. After receiving the injection, patients were positioned supine at hip height and instructed to rest on the examination bed for 15 to 20 min. The perfusion method of G-CSF was the same as that of the HCG group. Another physician reviewed the results of endometrium and hormone infusion to ensure accuracy.

Measurement of Endometrial Thickness, Distribution of Blood Flow, and Parameters of Uterine Blood Flow

The endometrial thickness was measured on the 11th day before intrauterine perfusion of estradiol valerate supplementation during the artificial cycle of hormone replacement in the three groups. Additionally, the measurements were taken on the second day after intrauterine perfusion for progesterone conversion as well as the day of embryo transfer using GE color Doppler ultrasound. The distribution of endometrial blood flow before and after intrauterine perfusion was observed using transvaginal ultrasound. It was categorized as either deficient (below grade II: I^+, I, I^-) or rich (grade II and above II^-, II, II^+, III). After measuring these indexes, the blood flow signals in the basal areas of the anterior and posterior walls were detected, and uterine blood flow parameters such as PI, RI, and S/D were obtained.

Endometrial Receptivity Measurement

4 ml of venous blood was taken from the patients before and after intrauterine perfusion, and 2 ml of serum was routinely separated. After this, the level of integrin αvβ3 was determined using Enzyme-Linked Immunosorbent Assay (ELISA) (15) with the Thermo Feil automatic enzyme marker.

The mononuclear cells were isolated from 2ml blood samples, and the proteins of peripheral blood mononuclear cells were extracted and added to a 2×SDS buffer solution. After electrophoresis, membrane transfer, mold removal, fixation, and closure, the osteopontin diluted in TBST was added. Following incubation for 24 hours, the osteopontin expression was detected by adding a secondary antibody, and using 3, 3-diaminobenzidine for color rendering (16).

Assessment of the Frequency of Regulatory T Cells in the Peripheral Blood Mononuclear Cells

Two milliliters of venous blood were collected from each patient before and after intrauterine perfusion. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples using density gradient centrifugation. The PBMC layer is collected for analysis. Cells were then stained

with a panel of fluorochrome-conjugated antibodies specific to Treg markers. In this study, antibodies against CD4 (a marker for T helper cells), CD25 (the interleukin-2 receptor alpha chain), and FoxP3 (a transcription factor specific to regulatory T cells) were used. The stained PBMCs were suspended in a fluid buffer and acquired on the flow cytometer. In this study, cells expressing CD4, CD25, and FoxP3 were identified as Tregs. The software calculates the percentage of Tregs (CD4+CD25++FoxP3+ cells) within the total PBMC population, providing a quantitative measure of Treg levels before and after intrauterine perfusion (17, 18).

Pregnancy Outcome

Secondary pregnancy outcomes, such as biochemical pregnancy rate, clinical pregnancy rate, and abortion rate, as well as main pregnancy outcomes such as full-term birth rate and preterm birth rate were recorded for the three groups. Biochemical pregnancy was defined as blood β-HCG levels≥20mIU/ml 2 weeks after embryo transfer, while clinical pregnancy was confirmed by the presence of a gestational sac on ultrasound examination 3 weeks after embryo transfer.

Statistical Analysis

SPSS22.0 statistical software was used for analysis and processing. Kolmogorov-Smirnov test data were employed to evaluate the normal distribution. One-way ANOVA was used for measurement data represented by LSD-t test was utilized for pairwise comparison between groups, and repeated measurement data before and after uterine perfusion were subjected to repeated measurement ANOVA. $\bar{x} \pm s$ If the data did not follow a normal distribution, M (Qn) was used after natural logarithm conversion to indicate non-parametric testing. Count data were presented as frequency and percentage, and group comparisons s were conducted using a chi-square^2 test. P<0.05 was considered statistically significant.

RESULTS

Comparison of the Demographic and Laboratory Data among the Three Groups of Patients

There were no significant differences in age, infertility duration, BMI, basal estradiol, basal follicle-stimulating hormone, and basal luteinizing hormone among the three groups (P>0.05, Table 1).

Comparison of Endometrial Thickness

As shown in Fig. 1, there was no significant difference in endometrial thickness among the three groups before intrauterine perfusion (P>0.05). However, after intrauterine perfusion, the endometrial thickness in all three groups significantly increased (P=0.037). After intrauterine perfusion, the endometrial thickness in the HCG and G-CSF groups was significantly thicker than that in the control group (P=0.015). Additionally, the endometrial thickness in the G-CSF group was higher than that in the HCG group (P=0.022). The endometrial thickness on embryo transfer day was significantly higher in the HCG and G-CSF groups compared to the control group (P=0.031). The intima thickness was higher in the G-CSF group than in the HCG group (P=0.019).

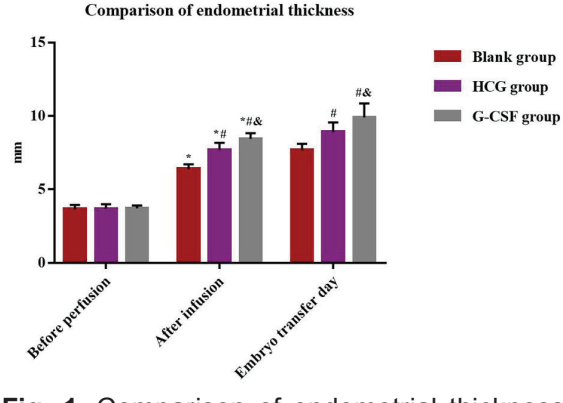

Fig. 1. Comparison of endometrial thickness before and after intrauterine perfusion as well as endometrial thickness on embryo transfer day among the three groups. Compared to before intrauterine perfusion, *P<0.05; Compared to the control group, #P<0.05; Compared to the HCG group, &P<0.05.

Comparison of Endometrial Blood Flow Distribution

There was no significant difference in endometrial blood flow distribution before intrauterine perfusion among the three groups (P>0.05). However, after intrauterine perfusion, the rate of blood deficiency decreased, and the rate of blood richness significantly increased compared to the levels before intrauterine perfusion (P<0.05). After intrauterine perfusion, the rate of blood flow deficiency in the HCG and G-CSF groups was lower than that in the control group, while the rate of blood flow richness was higher (P<0.05). The rate of blood flow deficiency in the G-CSF group was lower than that in the HCG group after intrauterine perfusion, and the rate of blood flow enrichment in the G-CSF group was higher than that in the HCG group (P<0.05, Fig. 2).

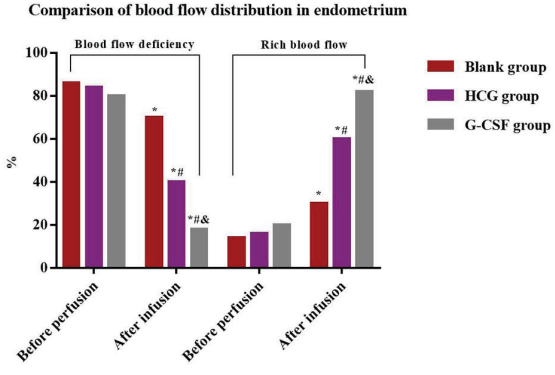

Fig. 2. Comparison of endometrial blood flow distribution before and after intrauterine perfusion among the three groups. Compared with before intrauterine perfusion, *P<0.05; Compared tothe control group, #P<0.05; Compared to the HCG group, &P<0.05.

Comparison of Uterine Blood Flow Parameters

There was no significant difference in uterine blood flow parameters before intrauterine perfusion among the three groups (P>0.05). However, after intrauterine perfusion, the uterine blood flow parameters PI, RI, and S/D show a decrease compared to before (P<0.05). The uterine blood flow parameters PI, RI, and S/D in the HCG and G-CSF groups after uterine perfusion were lower than those in the control group (P<0.05). Moreover, the uterine blood flow parameters PI, RI, and S/D in the G-CSF group after uterine perfusion were lower than those in the HCG group (P<0.05, Fig. 3).

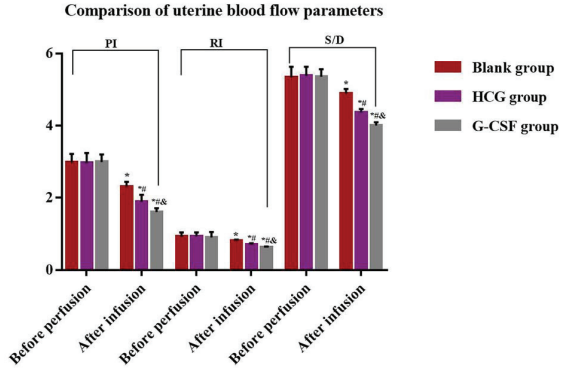

Fig. 3. Comparison of uterine blood flow parameters before and after intrauterine perfusion in the three groups. Compared to before intrauterine perfusion, *P<0.05; Compared to the control group, #P<0.05; Compared to the HCG group, &P<0.05.

Comparison of Endometrial Receptivity

There was no significant difference in endometrial receptivity among the three groups before intrauterine perfusion (P>0.05). However, following intrauterine perfusion, there was an increase in the endometrial receptivity indexes, integrin αvβ3 and osteopontin levels, compared to their levels before perfusion (P<0.05). After intrauterine perfusion, the levels of endometrial receptivity indexes integrin αvβ3 and osteopontin in the HCG and G-CSF groups were higher than those in the control group (P<0.05). In addition, the levels of endometrial receptivity indexes integrin αvβ3 and osteopontin in the G-CSF group after intrauterine perfusion were higher than those in the HCG group (P<0.05, Fig. 4).

Assessment of the Frequency of Regulatory T Cells in the Peripheral Blood Mononuclear Cells

There was no significant difference in the frequency of Treg cells in the peripheral blood before intrauterine perfusion among the three groups (P>0.05). However, the frequency of Treg cells in the peripheral blood increased after intrauterine perfusion (P<0.05).

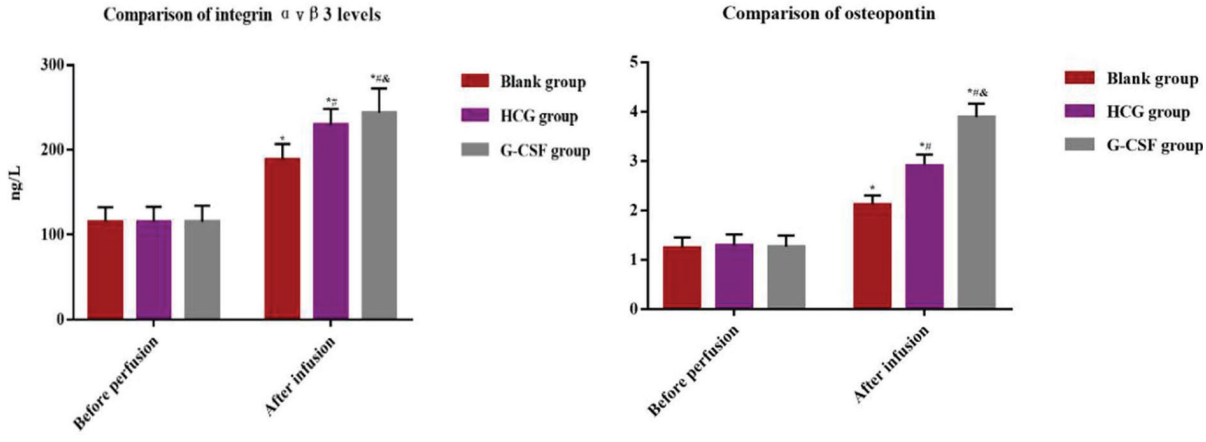

Fig. 4. Comparison of endometrial receptivity indexes before and after intrauterine perfusion among the three groups. Compared with before intrauterine perfusion, *P<0.05; Compared to the control group, #P<0.05; Compared to the HCG group, &P<0.05.

The frequency of Treg cells in the peripheral blood of the HCG and G-CSF groups after intrauterine perfusion was higher than that of the control group (P<0.05). The frequency of Treg cells in the peripheral blood of the G-CSF group after intrauterine perfusion was higher than that of the HCG group (P<0.05, Fig. 5).

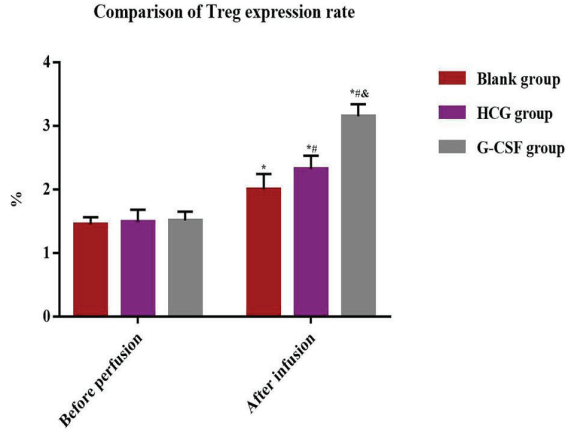

Fig. 5. Comparison of the frequency of peripheral blood Tregs before and after intrauterine perfusion among the three groups. Compared to before intrauterine perfusion, *P<0.05; Compared to the control group, #P<0.05; Compared to the HCG group, &P<0.05.

Comparison of Pregnancy Outcomes

The biochemical pregnancy rate and clinical pregnancy rate of the HCG and G-CSF groups were higher than those of the control group (P<0.05). The biochemical pregnancy rate and clinical pregnancy rate of the G-CSF group were higher than those of the HCG group (P<0.05). There was no significant difference in the abortion rate, preterm birth rate, and full-term birth rate among the three groups (P>0.05, Fig. 6).

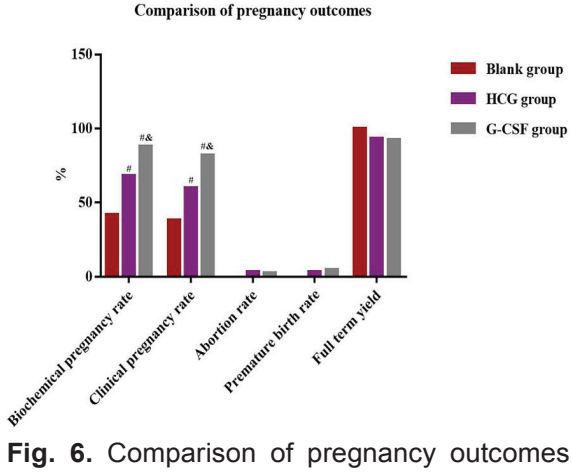

Fig. 6. Comparison of pregnancy outcomes among the three groups. Compared to the control group, #P<0.05; Compared to the HCG group, &P<0.05.

DISCUSSION

Endometrial thinning caused by many factors like endometrial lesions and multiple uterine operations, can decrease the pregnancy success rate of frozen-thawed embryo transfer. HCG content crucial for implantation, is closely linked to the rate of successful embryo implantation (19). There are varying degrees of HCG loss during the transfer cycle of frozen-thawed embryos. Studies

have shown that exogenous HCG can rapidly increase intrauterine HCG concentration (20). One report showed that (21-23) intrauterine perfusion of HCG can directly impact the endometrium, stimulating various factors related to endometrial implantation. This can change the receptivity by affecting endometrial blood flow distribution, ultimately promoting embryo implantation. In this study, the HCG group underwent intrauterine infusion of HCG on the day of progesterone conversion. The results showed that compared to patients who did not receive intrauterine perfusion drug intervention, those who received intrauterine HCG perfusion experienced a shift in uterine blood flow from insufficient to abundant, along with a significant increase in endometrial thickness. This result suggests that HCG could improve endometrial morphology and increase the endometrial thickness, similar to the findings of Mo Fengmei et al (24).

It is well known that G-CSF is a hematopoietic-specific cytokine secreted by stromal cells and bone marrow cells. However, it has been less recognized that G-CSF can affect a variety of mature cells in addition to promoting the proliferation and differentiation of bone marrow hematopoietic cells. It also plays a role in female reproduction. G-CSF has been confirmed to be involved in follicular growth and development, ovarian stimulation, pregnancy, and other reproductive processes (25). Research shows (26, 27) that the expression of G-CSF and G-CSF receptors in the human endometrium and the maternal-fetal interface remains high throughout pregnancy. Due to the special role of G-CSF in the female reproductive system, some scholars are currently dedicated to studying uterine perfusion of G-CSF on the outcome of embryo transfer. They believe that (28-30), on one hand, intrauterine perfusion of G-CSF can help treat endometrial inflammation and promote the repair and growth of the endometrium. On the other hand, it can stimulate the formation and maturation of leukocytes in the vascular network of the endometrium, showing a non-infectious inflammatory state, promoting the secretion of a variety of proteases in the endometrium, and affecting the molecular structure and function of the endometrial epithelial cells. This can increase the adhesion ability between the early embryo and the endometrium, ultimately facilitating embryo implantation. Lian et al. (31) found that patients undergoing frozen-thawed embryo transfer experienced a significant increase in endometrial thickness following intrauterine perfusion of G-CSF. In this study, the group receiving G-CSF was infused intrauterinely with this hormone on the day of progesterone conversion. Endometrial receptivity indexes are closely related to the endometrial environment. For example, Integrin αVβ3, an essential factor in the endometrium of implanting mouse embryos, is expressed in mouse blastomeres during the "window of implantation" period. It regulates the implantation process by acting on the adhesion and expansion pathways of the trophoblastic layer of the blastocyst to the uterine epithelium. Integrin αvβ3 plays a critical role in embryo implantation by mediating cell-cell and cell-extracellular matrix interactions, which are essential for the attachment and subsequent invasion of the embryo into the endometrial stroma (32). Studies have demonstrated that a lack of αvβ3 expression correlates with lower implantation rates and poorer pregnancy outcomes, highlighting its importance in reproductive success (33). On the other hand, osteopontin functions as a bridging molecule that interacts with αvβ3 and other integrins to promote cell adhesion, signaling, and tissue remodeling necessary for implantation. It is also involved in modulating the local immune environment, which is crucial for embryo tolerance and successful implantation (34). The results showed that compared with the control group and the HCG group, patients with G-CSF intrauterine perfusion had a more significant increase in endometrial thickness, more abundant blood flow, lower values of PI, RI, and S/D, as well as increased levels of endometrial receptivity indexes

integrin αvβ3 and osteopontin. These results indicatethat compared to patients who did not receive drug or HCG infusion, G-CSF intrauterine perfusion has more advantages in improving the endometrial environment of patients. The reason may be related to the fact that G-CSF could regulate the expression of cytokines between endometrial cells and trophoblasts, increase endometrial tolerance, and provide support for successful embryo transplantation, which is consistent with the results reported above.

Other studies have shown that (35-37) the success of embryo transfer depends on the regulation of the immune system. During the process of embryo transfer, the mother will experience immune rejection and an imbalance in immune tolerance towards the embryo. When this imbalance occurs, it can reduce the success rate of embryo implantation. Additionally, during this process, the frequency of Tregs decreases. The success of embryo transfer is affected by the degree of immune tolerance induced by Treg (38). The immune system plays a crucial role in pregnancy, where a delicate balance between immune activation and tolerance is required to support embryo implantation and fetal development. In patients with thin endometrium, immune defects can contribute to lower implantation success rates. Specifically, Tregs are essential in establishing immune tolerance by suppressing maternal immune responses against the semi-allogeneic fetus (39). Our study demonstrated that intrauterine perfusion of G-CSF significantly increased the frequency of Tregs in peripheral blood compared to the control and HCG groups. This suggests that G-CSF may help correct immune tolerance imbalances in patients with thin endometrium, enhancing the uterine environment for embryo implantation. G-CSF also exerts immunomodulatory effects within the reproductive system. It promotes angiogenesis and immune regulation, influencing endometrial cell function and enhancing endometrial receptivity (40). The increased frequenvy of Tregs observed in the G-CSF group could be due to G-CSF's ability to stimulate leukocyte formation and maturation within the endometrial vascular network, creating a non-infectious inflammatory state that favors embryo implantation. HCG, on the other hand, has been shown to modulate the immune environment indirectly by altering the secretion of estradiol and cytokines that affect endometrial receptivity (41). However, our results indicated that while HCG improved endometrial thickness and blood flow, its effect on Tregs was less pronounced compared to G-CSF, suggesting a more limited role in enhancing immune tolerance.

This study utilized a strong randomized control design involving three groups: a control group with no intervention, an HCG group, and a G-CSF group. Endometrial parameters and Treg levels were closely monitored before and after intrauterine perfusion. The use of transvaginal ultrasound and flow cytometry provided precise measurements of endometrial thickness, blood flow, and immune cell expression, ensuring reliable data. Our findings reveal that G-CSF is more effective than HCG in improving pregnancy outcomes for patients with thin endometrium, likely due to its dual role in enhancing endometrial receptivity and modulating immune tolerance. The increased frequency of Treg cellsand improved endometrial environment suggest that G-CSF facilitates a more favorable setting for embryo implantation, addressing a critical defect in immune tolerance mechanisms in these patients. Further studies should explore the molecular pathways by which G-CSF enhances Treg level and other aspects of immune tolerance in the endometrium. Understanding these mechanisms could lead to more targeted therapies for patients with thin endometrium and recurrent implantation failure, ultimately improving outcomes in assisted reproductive technology outcomes.

However, there are still some limitations in this study, such as being conducted as a single center, having a small sample size, and lacking

an in-depth analysis of the specific mechanism by which G-CSF intrauterine perfusion improves the pregnancy rate of freeze-thaw embryo transfer in thin endometrium. Further multi-center and large-sample in-depth analysis is needed in the future to clarify the role of G-CSF in the freeze-thaw embryo transfer in thin endometrium.

CONCLUSIONS

Compared to uninfused drugs and intrauterine perfusion of HCG, intrauterine perfusion of G-CSF is more beneficial for embryo implantation in patients with thin endometrium undergoing freezing-thawing embryo transplantation. G-CSF can change the endometrial blood flow distribution, improve endometrial tolerance, increase endometrial thickness, enhance Treg level, induce immune tolerance, facilitate embryo implantation, and improve pregnancy outcome.

AUTHORS' CONTRIBUTION

HJY was the guarantor of the integrity of the entire study and carried out study design, statistical analysis, manuscript preparation, and editing; XYL carried out study concepts, experimental studies, and manuscript review; QW contributed to literature research and data analysis; LT contributed to clinical studies and data acquisition. All authors have reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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