

Systematic Review

The Effect of Granulocyte Colony Stimulating Factor (G-CSF) on the Outcome of Assisted Reproduction Technology in Thin Endometrium Women: A Meta-Analysis

Li-Jun Lin^{1,2,3,†}, Ge Chen^{1,2,3,†}, Liang-Zhi Xu^{1,2,3}, Hui Chen^{1,2,3,*}¹Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, 610041 Chengdu, Sichuan, China²Reproductive Endocrinology and Regulation Laboratory, West China Second University Hospital, Sichuan University, 610041 Chengdu, Sichuan, China³Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, West China Second University Hospital, Sichuan University, 610041 Chengdu, Sichuan, China*Correspondence: chenhui2022@scu.edu.cn (Hui Chen)

†These authors contributed equally.

Academic Editor: Paolo Ivo Cavoretto

Submitted: 15 October 2022 Revised: 18 December 2022 Accepted: 6 January 2023 Published: 19 April 2023

Abstract

Background: To explore the effect of granulocyte colony-stimulating factor (G-CSF) on thin endometrium in women undergoing assisted reproduction. **Methods:** We performed a methodical search from their inception to December 2022 in various electronic databases containing PubMed, Cochrane Library, Embase, Web of Science, Scopus, in addition to a manual search. All journals concerning the effect of G-CSF on thin endometrium were found. Selected studies, collected data, and assessed risk of bias were conducted by two investigators under precise inclusion and exclusion criteria independently. We applied Revman 5.3 software to accomplish the Meta-analysis of qualified studies. **Results:** This research included 8 studies, including 6 randomized controlled trials and 2 non-randomized controlled studies, a total of 673 patients. Based on the meta-analysis, we noted that compared with the control group, G-CSF significantly improved embryo implantation rate [risk ratio (RR) = 1.91, 95% confidence interval (CI) (1.26, 2.91), $p = 0.002$] and clinical pregnancy rate [RR = 1.73, 95% CI (1.22, 2.45), $p = 0.002$]. Compared with the control group, the endometrial thickness in the G-CSF group had non-significant increase compared with that of the control group [mean difference (MD) = 0.81, 95% CI (−0.04, 1.67), $p = 0.06$], in randomized controlled trial (RCT) studies, subgroup analysis shows G-CSF group increased significantly [MD = 1.13, 95% CI (0.56, 1.67), $p < 0.0001$]. **Conclusions:** G-CSF may improve implantation rate and clinical pregnancy rate in assisted reproductive technology, and have the potential to increase the endometrial thickness among women with thin endometrium. However, the results of the included studies should be explained with caution due to their limited quantity and quality, and more studies of higher quality are demanded to prove these findings.

Keywords: granulocyte colony stimulating factor; thin endometrium; assisted reproductive technology; meta-analysis

1. Introduction

Infertility is assessed to affect approximately 8–12% of husband and wife of childbearing age worldwide [1]. Assisted reproductive technology (ART) has been widely used in the treatment of infertility over the past few decades. In 2014, about 1.93 million ART cycles were carried out in 76 countries around the world, frozen embryo transfer cycles increased by about 2/3 compared with 2010 [2]. High quality embryos and endometrial receptivity are both critical for a successful pregnancy; however, endometrial receptivity may represent a key rate-limiting factor for pregnancy rates in ART. An important marker of endometrial receptivity is endometrial thickness; a thin endometrium is closely associated with a poor pregnancy outcome after embryo transfer [3–5]. In ART, a thin endometrium is often defined as an endometrial thickness <7 mm; this condition affects 1.3–2.3% of women undergoing fertility treatment [5–7]. Treating patients with a thin endometrium is a crucial challenge for patients undergoing ART.

Clinically, there are several different approaches that considered to be helpful to treat patients with a thin endometrium, including aspirin, human chorionic gonadotropin, sildenafil citrate, estrogen, antioxidant, vitamin E, alpha-tocopherol, pentoxifylline, nifedipine and so on [8]. These treatments are selected based on the type of embryo transfer (ET) being planned. Nonetheless, embryo transfer cycles must be cancelled repeatedly due to some patients' endometrium is still unresponsive after using these therapies, even embryo transferred may fail in implantation [8].

Granulocyte colony stimulating factor (G-CSF) is a cytokine that belongs to a large family of cytokines [9]. Previous studies have shown that G-CSF act vital function in endometrial growth and pregnancy. G-CSF may be involved in embryo implantation by regulating decidual macrophages and Th2 reaction [10]; G-CSF can induce the proliferation of endometrial epithelium and stromal cells by increasing the staining level of proliferating cell nuclear



antigen, and promote the regeneration of thin endometrium [11]; G-CSF shown to stimulate the directed differentiation of stem cells at the site of injury and increase the thickness of the endometrium in rat models [11]. Therefore, G-CSF may be an effective method to treat thin endometrium. Several clinical studies have investigated the use of G-CSF to treat women with a thin endometrium [12–16]. However, inconsistent results have been reported with regards to the influence of intrauterine G-CSF infusion on pregnancy rate in clinical studies. Some studies have reported an improvement in pregnancy rate [12–14] while others did not [15,16]. Therefore, in the present study, we conducted a meta-analysis to investigate the effect of G-CSF on thin endometrium during ART in the hope that this may provide useful guidance on how to prepare the endometrium for embryo transfer in patients with a thin endometrium.

2. Materials and Methods

2.1 Study Selection

2.1.1 Inclusion Criteria

(1) The style of the study was a randomized controlled trial (RCT) or non-RCT. (2) Study subjects were patients whose endometrial thickness was <7 mm during ART. (3) Intervention measures in the treatment group involved the intrauterine infusion or subcutaneous application of G-CSF while the intervention measures in the control group involved the intrauterine infusion of normal saline or a blank control. (4) The outcomes of the study included embryo implantation rate, clinical pregnancy rate, and/or endometrial thickness on the day of embryo transfer. (5) The studies were published and contained sufficient information on sample size and research results.

2.1.2 Exclusion Criteria

(1) The style of the study was an observational study, descriptive study, retrospective study, case report or review. (2) The study subjects were animals. (3) The baseline of the treatment group and the control group was inconsistent. (4) Studies involved a defective design or inappropriate statistical methods and could not be corrected. (5) Duplicate studies.

2.2 Method

2.2.1 Search Strategy

We searched PubMed, the Cochrane Library, Embase, Scopus and Web of Science from their inception to December 2022; we also conducted a manual search. The search strategy was as follows: (“Reproductive Techniques, Assisted”[Mesh]) OR (assisted reproducti*) OR (IVF) OR (*in vitro* fertili*) OR (ICSI) OR (intracytoplasmic sperm injection) OR (embryo* AND transfer*) OR (blastocyst* AND transfer*) OR (FET) OR (ET) OR (Embryo Transfer) OR (ovarian stimulation) OR (ovulation induction) OR (controlled ovarian hyperstimulation) OR (COH)) AND ((thin* endometri*) OR (endometri* thin*) OR (endometri* thick*)) AND (“Granulo-

cyte Colony-Stimulating Factor”[Mesh]) OR (Granulocyte Colony Stimulating Factor) OR (GCSF) OR (neupogen) OR (filgrastim) OR (pegfilgrastim) OR (lenograstim) OR (molgramostim) OR (sargramostim)) (Appendix Table 3).

2.2.2 Data Extraction

Two reviewers carried out articles selected and data extracted independently. First, the reviewers assessed relevant studies by reading the title and abstract. Then, they read through the entire study to decide whether it met the inclusion criteria. Next, the two reviewers compared their results; if they disagreed, they would take advice from an expert in the field who acted as a third reviewer. Finally, the reviewers acquired data from each selected study based on a pre-prepared data collection table. The corresponding author was contacted to obtain data whenever there was an incomplete dataset. The reviewers collated a range of data: (1) basic data, including the first author’s name and publication date; (2) study design; (3) factors used to assess the risk of bias; (4) essential patient characteristics, including the size of the treatment and control groups, nation, patient age and baseline endometrial thickness; (5) information relating to intervention strategies including the timing, method, dose and frequency of intrauterine G-CSF infusion; (6) outcome indicators, including clinical pregnancy rate, embryo implantation rate and endometrial thickness.

2.2.3 Bias Risk Assessment

The risk of bias for the RCTs included in this meta-analysis was evaluated by the Cochrane risk-of-bias tool [17]. We also employed the methodological index for non-randomized studies (MINORS) to evaluate the non-RCTs bias risk [18].

2.3 Statistical Analysis

The meta-analysis was carried out with Review Manager 5.3 (Cochrane Collaboration, Copenhagen, Denmark) [19]. We used mean differences (MD) to describe continuous variables and relative risk (RR) to show categorical variables. We calculated point estimates and 95% confidence intervals (95% CIs) for each outcome measure. The meta-analysis was performed with random and fixed effects models according to the I^2 statistic. Funnel plots were not generated because only 8 studies were included in this meta-analysis.

3. Results

3.1 Study Selection

Following careful selection and consideration of the inclusion and exclusion criteria, 8 studies were finally selected for the meta-analysis, including 6 RCTs [12–14,20–22] and 2 non-RCTs [15,16]. A total of 673 patients were included in our research. Fig. 1 shows the number of selected studies and the reasons for exclusion at each step.

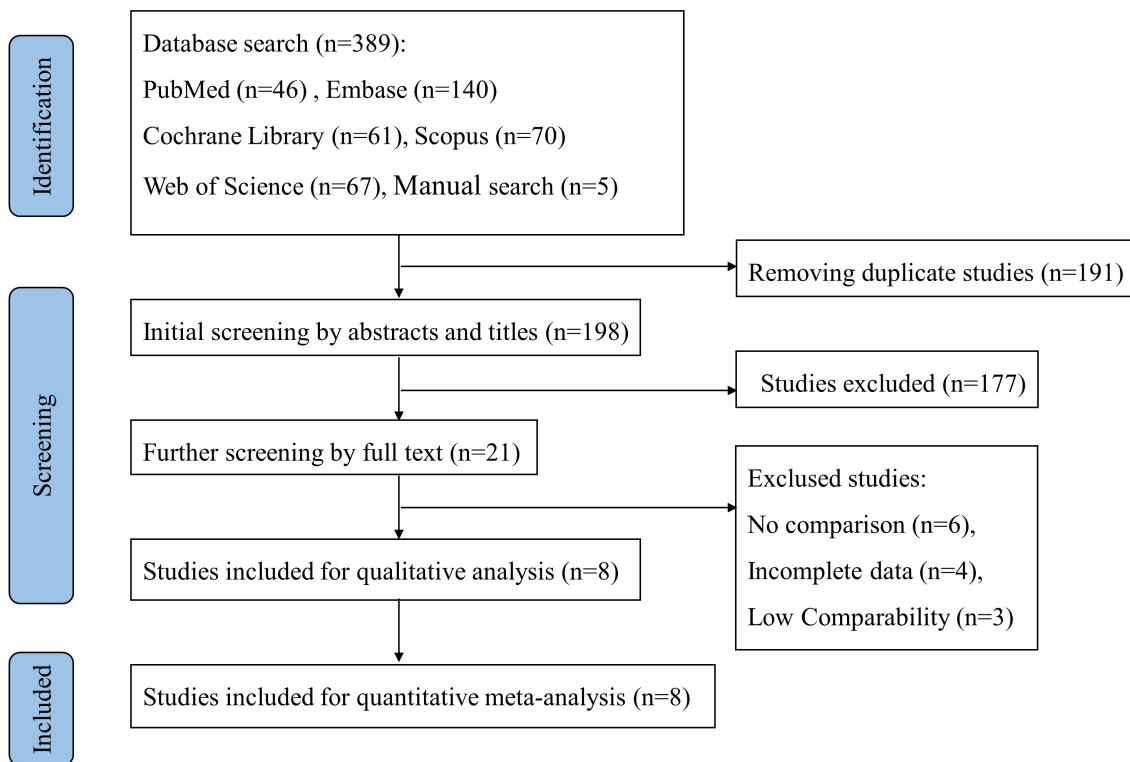


Fig. 1. Flow chart showing study selection.

3.2 Basic Characteristics and Risk of Bias Assessment

The characteristics of the included studies are presented in Table 1 (Ref. [12–16,20–22]), including the number of patients, nation, mean age, baseline endometrial thickness, interventions, and outcome measures for each study. These studies covered 6 countries (2 in Iran, 2 in China, and each in Portugal, Spain, Poland, India). A total of 8 eligible studies were included in this meta-analysis. 5 studies reported the embryo implantation rate, 7 eligible studies reported the clinical pregnancy rate, 4 studies reported endometrial thickness.

An assessment for the risk of bias for the included RCTs is shown in Figs. 2,3. RCTs [13,20,22] applied a random sequence generation process to avoid selection bias; one of these RCTs [13] also described the use of a ‘sealed envelope’ to ensure allocation concealment. 4 RCTs [12–14,22] described the blinding of participants and personnel, and 5 RCTs [12–14,21,22] described the blinding of outcome assessments. However, 2 RCTs [12,14] had an incomplete dataset for outcomes. 6 RCTs [12–14,20–22] did not describe selective reporting and other forms of bias. All RCTs were considered to represent medium risk.

Table 2 (Ref. [15,16]) shows the results derived from the assessment of methodological quality of non-RCTs. Analysis showed that the non-RCTs scored 22 (more than 12 points) and were considered to represent a low risk of bias.

3.3 Meta-Analysis Results

3.3.1 Embryo Implantation Rate

A total of 5 studies reported the embryo implantation rate [12–14,16,20]. A fixed-effects model was employed when statistical results were homogeneous ($p = 0.87$, $I^2 = 0\%$). Meta-analysis revealed a significantly higher embryo implantation rate in the G-CSF group when compared with the control group [RR = 1.91, 95% CI (1.26, 2.91), $p = 0.002$] (Fig. 3). Sub-group analysis was conducted for the included 4 RCTs [12–14,20] and a fixed-effect model was used because of adequate homogeneity among these RCTs ($p = 0.95$, $I^2 = 0\%$). Sub-group analysis showed that when compared with the control group, the clinical pregnancy rate of the G-CSF group was significantly higher [RR = 2.08, 95% CI (1.33, 3.26), $p = 0.001$] (Fig. 4).

3.3.2 Clinical Pregnancy Rate

All 7 eligible studies were involved when considering clinical pregnancy rate [12–16,20,21]. We used a fixed-effect model because there was adequate homogeneity among studies ($p = 0.81$, $I^2 = 0\%$). The clinical pregnancy rate was significantly higher in the G-CSF group than that in the control group [RR = 1.73, 95% CI (1.22, 2.45), $p = 0.002$] (Fig. 5). Sub-group analysis was performed for the included 5 RCTs [12–14,20,21], in view of there was sufficient homogeneity across these RCTs, a fixed-effect model was applied ($p = 0.80$, $I^2 = 0\%$). The clinical pregnancy rate in the G-CSF group was also significantly higher when

Table 1. Basic characteristics of included studies.

Study	Patients No. (T/C)	Mean age (T/C, years)	Baseline EM (mm) (T/C)	EM after intervention	Interventions (T/C)	Outcome measures
Singh R 2015 [12]	48 (24/24)	<42	6.49 ± 1.65/NA	8.79 ± 1.57/NA	Intrauterine infusion of 300 µg/1 mL G-CSF on the day of hCG administration (second infusion following oocyte retrieval in subgroup patients) vs. intrauterine perfusion of placebo (1 mL saline solution)	IR, CPR
Sarvi F 2017 [13]	28 (15/13)	31.6 ± 3.8/31.2 ± 3.2	4.1 ± 1.8/4.2 ± 1.6	9.1 ± 1.5/6.9 ± 1.1	Intrauterine infusion of 300 µg/1 mL G-CSF on the day of hCG administration; if EM was less than 6 mm, a second dose of G-CSF was injected 2–3 days after oocyte retrieval day in 3/15 patients vs. intrauterine perfusion of placebo (1 mL saline solution)	EM, IR, CPR
Singh R 2018 [14]	112 (56/56)	NA	6.23 ± 1.45/NA	8.46 ± 1.27/NA	Intrauterine infusion or subcutaneous injection of G-CSF 300 µg/1 mL on the day of hCG administration; a second dose of G-CSF after oocyte retrieval day in some patients vs. placebo (intrauterine perfusion of saline solution or subcutaneous injection of decavitamin)	IR, CPR
Eftekhari M 2014 [15]	68 (34/34)	30.81 ± 4.60/28.57 ± 5.16	5.63 ± 0.78/5.76 ± 0.86	7.91 ± 0.55/8.23 ± 0.82	Intrauterine infusion of 300 µg/1 mL G-CSF at the 12th–13th day of frozen-thawed embryo transfer cycle, a second dose of G-CSF was given 2–3 day after first infusion in 6/34 patients vs. blank control	EM, CPR
Kunicki M 2017 [16]	62 (29/33)	38 (32.75–41.25)/37 (35–40)	6.50 (5.50–6.80)/6.40 (5.50–7.00)	7.90 (6.58–8.70)/6.90 (6.0–7.75)	Intrauterine infusion of 300 µg/1 mL G-CSF at 9th day of frozen-thawed embryo transfer in endometrial preparation vs. blank control	IR, CPR
Xu B 2015 [20]	66 (14/52)	31.9 ± 4.1/32.0 ± 3.9	5.7 ± 0.7/6.5 ± 0.5	8.5 ± 2.4/NA	Intrauterine instillation of 300 µg G-CSF (100 µg/0.6 mL) on the day that one follicle became dominant (almost 12 × 12 mm in diameter) vs. blank control	IR, CPR
Jindal PC 2021 [21]	60 (30/30)	NA	5.9 ± 0.7/5.8 ± 0.6	7.9 ± 0.5/6.9 ± 0.4	Intrauterine infusion of 300 µg/1 mL G-CSF on day 14 of frozen-thawed embryo transfer cycle, a second infusion of G-CSF was carried out after 48 hours if endometrium was less than 7 mm vs. injection of G-CSF (300 mcg/1 mL) subcutaneously on day 14 onwards alternate days for two doses	EM, CPR
Zhang Y 2022 [22]	229 (114/115)	31.7 ± 4.3/32.2 ± 4.1	5.50 ± 1.96/5.68 ± 2.22	7.91 ± 2.12/7.22 ± 2.04	Intrauterine perfusion with 300 mg (1.8 mL) G-CSF vs. 1.8 mL normal saline	EM

Table footnotes: T, treatment group (G-CSF group); C, control group; hCG, human chorionic gonadotropin; NA, Not applicable; EM, endometrial thickness; IR, implantation rate; CPR, clinical pregnancy rate.

Table 2. Assessment of risk of bias for the included non-RCTs.

Study	Non-comparative study								Score
	A clearly stated aim	Inclusion of consecutive patients	Prospective collection of data	Endpoints appropriate to the aim of the study	Unbiased assessment of the study endpoint	Follow-up period appropriate to the aim of the study	Loss to follow-up less than 5%	Prospective calculation of the study size	
Eftekhar M 2014 [15]	2	2	2	2	2	2	2	0	/
Kunicki M 2017 [16]	2	2	2	2	2	2	2	0	/

Study	Additional criteria in the case of comparative study				Score
	An adequate control group	Contemporary groups	Baseline equivalence of groups	Adequate statistical analyses	
Eftekhar M 2014 [15]	2	2	2	2	22
Kunicki M 2017 [16]	2	2	2	2	22

Table footnotes: The items are scored 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate).

compared with the control group in the subgroup analysis [RR = 1.69, 95% CI (1.15, 2.49), $p = 0.008$] (Fig. 6).

3.3.3 Endometrial Thickness

Four studies were included when considering endometrial thickness [13,15,21,22]. A random-effect model was adopted due to statistical heterogeneity across these studies ($p < 0.00001$, $I^2 = 94\%$). There was a non-significant increase in endometrial thickness in G-CSF treated patients when compared to the control group [MD = 0.81, 95% CI (–0.04, 1.67), $p = 0.06$] (Fig. 7). Subgroup analysis was conducted for 2 of the included RCTs [13,21,22] and a random-effect model was used because of statistical heterogeneity among these RCTs ($p = 0.03$, $I^2 = 71\%$). In the subgroup analysis, the G-CSF group had a significantly higher endometrial thickness when compared with the control group [MD = 1.13, 95% CI (0.56, 1.67), $p < 0.0001$] (Fig. 8).

4. Discussion

Successful assisted reproduction requires a receptive endometrium; however, studies have shown that the implantation rate is significantly lower in women with a thin endometrium [23]. A thin endometrium has an unfavorable effect on reproductive outcome. Various therapeutic methods have been used for patients with a thin endometrium. Although there are many types of treatments, most existing methods only achieve a slight change in endometrial thickness and pregnancy outcome. Consequently, it is very challenging to treat women with a thin endometrium when considering ART [24]. Previous studies have suggested that G-CSF may increase the embryo implantation rate and clinical pregnancy rate in women with a thin endometrium. G-CSF, as a type of cytokine, can affect bone marrow mesenchymal stem cells and decidual macrophages, thus influencing endometrial growth and development [25]. Using a rat model of thin endometrium, a previous study showed that the intrauterine infusion of G-CSF could improve endometrial receptivity by regulating endometrial hyperplasia and angiogenesis [26]. Zhao *et al.* [27] reported that G-CSF could mobilize stem cells to the site of injury and repair tissue in animal models, when compared with control groups, G-CSF resulted in obvious thickening of the endometrium along with increased expression levels of cytokeratin and vimentin. The intrauterine perfusion of G-CSF, as a chemical and mechanical stimulus, is considered to induce the secretion of endogenous cytokines and activate endocrine-paracrine pathways, thus leading to successful embryo implantation and pregnancy [15]. At the genetic level, G-CSF can downregulate the expression of hsa_circ_0001550, the downstream target genes of hsa_circ_0001550 participate in regulating endometrial receptivity and embryo implantation, so G-CSF may affect embryo implantation by adjusting hsa_circ_0001550-miRNA-mRNA interaction network [28]. Furthermore, by promoting embryonic adhesion, cell migration, tissue remodeling, and the expression

of angiogenesis-related genes, G-CSF may contribute to embryo implantation [29]. G-CSF has been shown to be involved in embryonic growth and development [30] and play a role in the maintenance of pregnancy [31].

Several clinical studies have investigated the use of G-CSF to treat women with a thin endometrium with G-CSF. In 2011, Gleicher *et al.* [30] were the first to report four infertile women with a thin endometrium who successfully became pregnant by ART following the intrauterine perfusion of G-CSF. Subsequently, the same research group recruited another cohort of patients with a persistent thin endometrium who had failed conventional treatment and underwent intrauterine G-CSF perfusion. These researchers found that these patients achieved a low but reasonable clinical pregnancy rate following the intrauterine infusion of G-CSF [32]. Since then, several studies have investigated the use of G-CSF to treat women with a thin endometrium. However, the results of these studies were inconsistent. For example, Mishra *et al.* [33] reported that G-CSF led to a slight increase in endometrial thickness in women with a persistent thin endometrium; however, G-CSF did not improve pregnancy rate in such patients. In another study, Swati *et al.* [34] found that G-CSF improved endometrial thickness only in about a third of women with a persistent thin endometrium. Kim *et al.* [35] reported that G-CSF improved the thickness of a thin endometrium without causing intrauterine adhesions and increased the chances of conception and embryo implantation. Due to such inconsistency, it was evident that we needed to perform a meta-analysis of the published studies to determine whether G-CSF can affect endometrial thickness and pregnancy.

In total, we investigated 8 studies (consisting of 6 RCTs and 2 non-RCTs including 673 patients) in this meta-analysis. We discovered that intrauterine infusion or the subcutaneous injection of G-CSF significantly increased embryo implantation and clinical pregnancy rates when compared with controls. The effect of G-CSF on clinical pregnancy rate and embryo implantation rate were consistent before and after subgroup analysis, irrespective of whether we were considering RCTs or not. The use of G-CSF did not have a significant effect on endometrial thickness. Three studies were used to analyze endometrial thickness, including two RCTs and one non-RCT. Based on the results of sub-group analysis for two RCT studies, we found that G-CSF did increase endometrial thickness. As we know women with thin endometrium may have a higher risk of preterm birth, low birth weight, and miscarriage, embryo transplantation in patients with thin endometrium should be cautious, delaying transplantation to the cycle of thickening before transplantation can be suggested [36]. Therefore, G-CSF may improve the pregnancy outcome by increasing the thickness of endometrium. However, only two RCTs were involved in this analysis; consequently, there is a significant need for more RCTs to fully validate our findings.

Jindal, P. C. 2021	?	?	?	?	?	?
Sarvi, F. 2017	+	+	+	+	+	+
Singh, R. 2015	?	?	?	?	?	?
Singh, R. 2018	?	?	?	?	?	?
Xu, B. 2014	+	?	+	+	+	+
Zhang, Y. 2022	+	+	+	+	+	+

+	Low risk	?	Some concern	-	High risk
---	----------	---	--------------	---	-----------

Fig. 2. Assessment of risk of bias for the included RCTs.

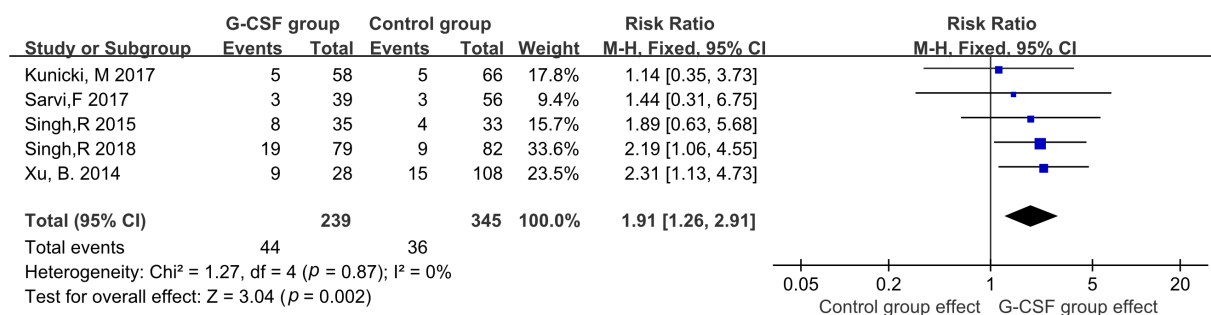


Fig. 3. Comparison of embryo implantation rate between G-CSF group and control group.

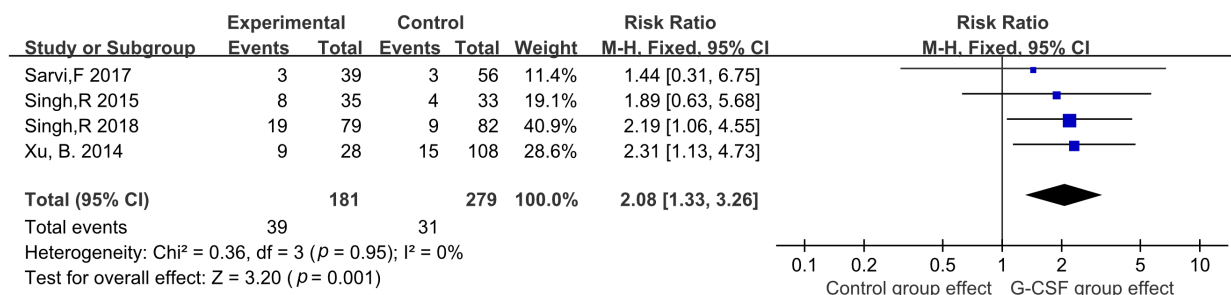


Fig. 4. Comparison of embryo implantation rate between G-CSF group and control group in RCTs.

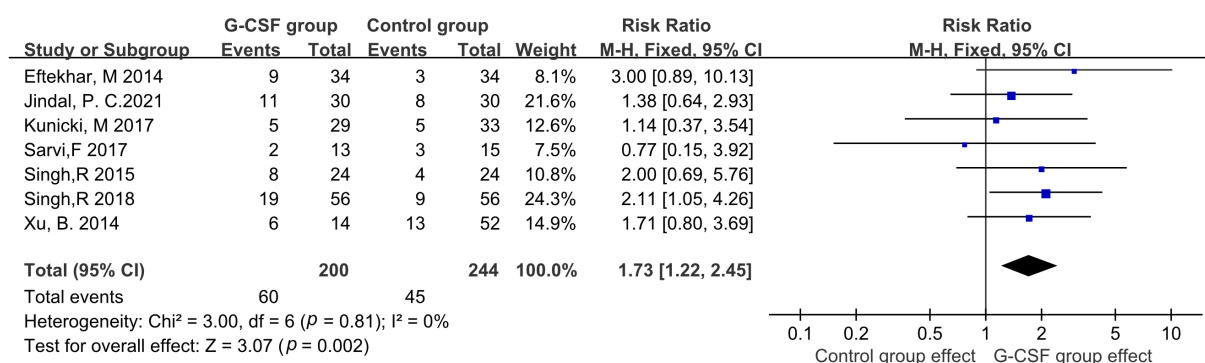


Fig. 5. Comparison of clinical pregnancy rate between G-CSF group and control group.

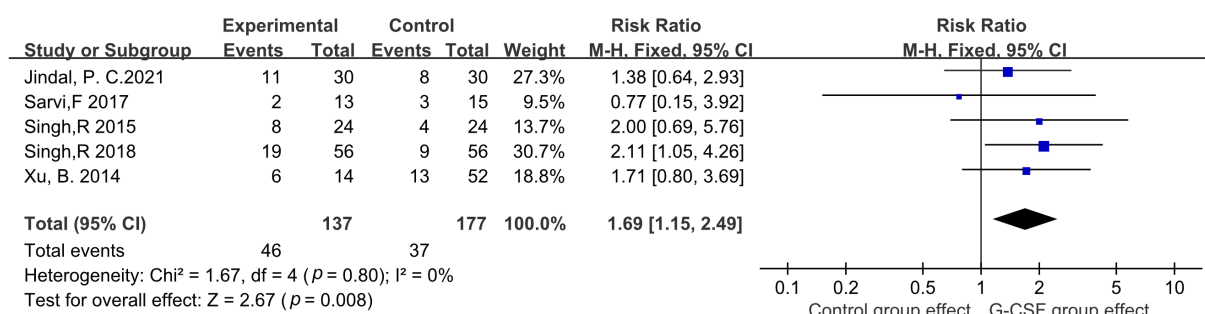


Fig. 6. Comparison of clinical pregnancy rate between G-CSF group and control group in RCTs.

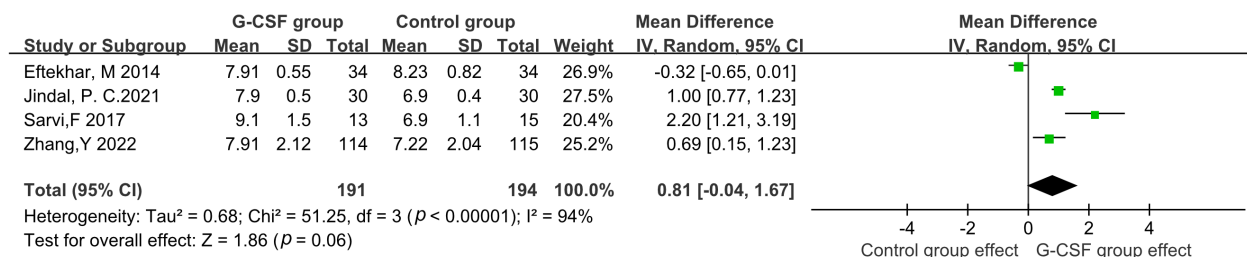


Fig. 7. Comparison of endometrial thickness between G-CSF group and control group.

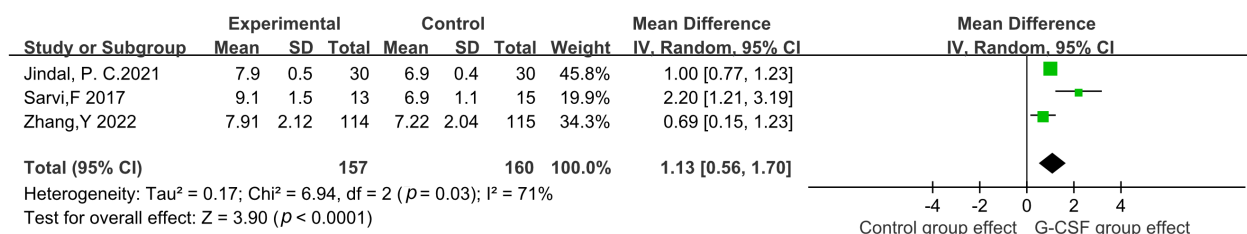


Fig. 8. Comparison of endometrial thickness between G-CSF group and control group in RCTs.

A systematic review and meta-analysis of the use of G-CSF in ART was previously published by Kamath *et al.* [37] in 2017; however, this study only included 1 non-RCTs

and 3 RCTs with low quality evidence. In the present meta-analysis, we included 8 studies, including 6 RCTs and 2 non-RCTs; there has been an increase in relevant research

over recent years and the level of evidence for the studies included in our study was better than the previous study [37]. These previous authors reported significantly a higher clinical pregnancy rate after the use of G-CSF when compared with controls [RR = 2.43, 95% CI (1.09, 5.40), $I^2 = 0\%$] but did not identify a statistically significant increase in endometrial thickness when G-CSF was used to treat women with a thin endometrium [MD = 0.47, 95% CI (1.36, 2.31), $I^2 = 82\%$] [37]. These conclusions are similar with our present findings and another meta-analysis conducted by Zhao *et al.* [38]. However, the study of Zhao *et al.* [38] analyzed collated data from studies with prospective and retrospective designs; this may have affected analytical efficiency.

There are several limitations to our current analysis that need to be considered. First, the sample size of our analysis was small, as only five RCTs and two non-RCTs were included; this may have led to bias. Despite the limited number of studies, when stratified by RCTs *vs.* non-RCTs, subgroup analyses related to embryo implantation rate and clinical pregnancy rate showed consistent results with those of the overall pooled analyses. Inconsistent results were detected with regards to the influence of G-CSF on endometrium thickness; therefore, further studies are now needed to clarify the true benefits of G-CSF treatment. Second, the dosage and methods of G-CSF administration, as well as the underlying causes of thin endometrium, tended to vary across the included studies. Since the available studies did not individualize treatment strategies according to different underlying causes of thin endometrium, it was impossible to generate a definitive conclusion with this dataset. Therefore, well-designed randomized controlled studies with a large sample size and high quality are still needed to validate our findings. Current studies are still in the exploratory stage and there is no uniform standard for dosage, the times of administration, route of administration and timing of administration.

5. Conclusions

The results of our meta-analysis indicate that G-CSF may increase embryo implantation rate and clinical pregnancy rate of patients with thin endometrium during ART, G-CSF may have the potential to increase the endometrial thickness, although further studies are needed to fully validate the impact of G-CSF on endometrial thickness. Nevertheless, our findings should be considered cautiously conclusion owing to the limited number and quality of the studies involved in our meta-analysis, as well as differences in the etiology of thin endometrium, the frequency and route of G-CSF administration. Large-scale, high-quality, multi-center RCTs are now needed to fully elucidate the benefits of G-CSF on thin endometrium.

Author Contributions

HC, L-JL and GC designed the study. L-JL, GC carried out the research and collated data. L-ZX interpreted the data of the study and supervised L-JL. All authors wrote and edited the manuscript and approved the final version. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

We appreciate Linbo-Gao who provided support during the writing of this manuscript.

Funding

This meta-analysis formed part of a ‘Study on the risk factor assessment tool for premature ovarian failure’ which is funded by the Technological Innovation and Development Program of Chengdu Bureau of Science and Technology (2019-YFYF-00101-SN); and was also part of ‘Creating a risk factor assessment tool for premature ovarian failure’, which was funded by the Key Science Research Program of Health Commission of Sichuan Province (20ZD008).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.ceog5004085>.

Appendix

See Table 3.

Table 3. Search strategy.

Cochranelibrary	
#1 MeSH descriptor: [Reproductive Techniques, Assisted] explode all trees MeSH	3583
#2(assisted reproducti*) OR (IVF) OR (<i>in vitro</i> fertili*) OR (<i>in-vitro</i> fertil*) OR (ICSI) OR (intracytoplasmic sperm injection) OR ((embryo* AND transfer*)) OR ((blastocyst* AND transfer*)) OR (FET) OR (ET) OR (Embryo Transfer) OR (ovarian stimulation) OR (ovulation induction) OR (controlled ovarian hyperstimulation) OR (COH)	110021
#3 #2 OR #3	110172
#4 (thin* endometri*) OR (endometri* thin*) OR (endometri* thick*)	2664
#5 MeSH descriptor: [Granulocyte Colony-Stimulating Factor] explode all trees MeSH	1559
#6 (Granulocyte Colony Stimulating Factor) OR (G-CSF) OR (GCSF) OR (neupogen) OR (filgrastim) OR (pegfilgrastim) OR (lenograstim) OR (molgramostim) OR (sargramostim)	7087
#7 #5 OR #6	7087
#8 #1 AND #6 AND #7	61
Embase	
#1 'reproductive techniques, assisted'/exp OR 'reproductive techniques, assisted' OR (assisted AND reproducti*) OR 'ivf'/exp OR ivf OR ' <i>in vitro</i> '/exp OR ' <i>in vitro</i> ' OR (in AND vitro AND fertili*) OR 'icsi'/exp OR icsi OR 'intracytoplasmic sperm injection'/exp OR 'intracytoplasmic sperm injection' OR (intracytoplasmic AND ('sperm'/exp OR sperm) AND ('injection'/exp OR injection)) OR (embryo* AND transfer*) OR (blastocyst* AND transfer*) OR fet OR et OR 'embryo transfer'/exp OR 'embryo transfer' OR (('embryo'/exp OR embryo) AND ('transfer'/exp OR transfer)) OR 'ovarian stimulation'/exp OR 'ovarian stimulation' OR (ovarian AND ('stimulation'/exp OR stimulation)) OR 'ovulation induction'/exp OR 'ovulation induction' OR (('ovulation'/exp OR ovulation) AND ('induction'/exp OR induction)) OR 'controlled ovarian hyperstimulation'/exp OR 'controlled ovarian hyperstimulation' OR (controlled AND ovarian AND ('hyperstimulation'/exp OR hyperstimulation)) OR coh	9389150
#2 'granulocyte colony-stimulating factor'/exp OR 'granulocyte colony-stimulating factor' OR 'granulocyte colony stimulating factor'/exp OR 'granulocyte colony stimulating factor' OR (('granulocyte'/exp OR granulocyte) AND ('colony'/exp OR colony) AND stimulating AND factor) OR gcsf OR 'neupogen'/exp OR neupogen OR 'filgrastim'/exp OR filgrastim OR 'pegfilgrastim'/exp OR pegfilgrastim OR 'lenograstim'/exp OR lenograstim OR 'molgramostim'/exp OR molgramostim OR 'sargramostim'/exp OR sargramostim	117670
#3 thin* AND endometri* OR (endometri* AND thin*) OR (endometri* AND thick*)	12997
#4 #1 AND #2 AND #3	140
PubMed	
#1 "Reproductive Techniques, Assisted"[Mesh] OR (assisted reproducti*) OR (IVF) OR (<i>in vitro</i> fertili*) OR (ICSI) OR (intracytoplasmic sperm injection) OR (embryo* AND transfer*) OR (blastocyst* AND transfer*) OR (FET) OR (ET) OR (Embryo Transfer) OR (ovarian stimulation) OR (ovulation induction) OR (controlled ovarian hyperstimulation) OR (COH)	10586096
#2 (thin* endometri*) OR (endometri* thin*) OR (endometri* thick*)	6033
#3 ("Granulocyte Colony-Stimulating Factor"[Mesh]) OR (Granulocyte Colony Stimulating Factor) OR (G-CSF)) OR (GCSF) OR (neupogen) OR (filgrastim) OR (pegfilgrastim) OR (lenograstim) OR (molgramostim) OR (sargramostim)	4,7057
#4 #1 AND #2 AND #3	46
Web of Science	
#1 TS=(Reproductive Techniques, Assisted) OR ALL=(assisted reproducti*) OR ALL=(IVF) OR ALL=(<i>in vitro</i> fertili*) OR ALL=(<i>in-vitro</i> fertil*) OR ALL=(ICSI) OR ALL=(intracytoplasmic sperm injection) OR ALL=((embryo* AND transfer*)) OR ALL=((blastocyst* AND transfer*)) OR ALL=(FET) OR ALL=(ET) OR ALL=(Embryo Transfer) OR ALL=(ovarian stimulation) OR ALL=(ovulation induction) OR ALL=(controlled ovarian hyperstimulation) OR ALL=(COH)	1425950
#2 ALL=(thin* endometri*) OR ALL=(endometri* thin*) OR ALL=(endometri* thick*)	8277
#3 TS=(Granulocyte Colony-Stimulating Factor) OR ALL=(Granulocyte Colony Stimulating Factor) OR ALL=(G-CSF) OR ALL=(GCSF) OR ALL=(neupogen) OR ALL=(filgrastim) OR ALL=(pegfilgrastim) OR ALL=(lenograstim) OR ALL=(molgramostim) OR ALL=(sargramostim)	70291
#4 #3 AND #2 AND #1	67
Scopus	
#1 TITLE-ABS-KEY("Reproductive Techniques, Assisted" OR (assisted AND reproducti*) OR (ivf) OR (in AND vitro AND fertili*) OR (icsi) OR (intracytoplasmic AND sperm AND injection) OR (embryo* AND transfer*) OR (blastocyst* AND transfer*) OR (fet) OR (et) OR (embryo AND transfer) OR (ovarian AND stimulation) OR (ovulation AND induction) OR (controlled AND ovarian AND hyperstimulation) OR (coh)	1888943
#2 TITLE-ABS-KEY (thin* endometri*) OR ALL=(endometri* thin*) OR ALL=(endometri* thick*)	9468
#3 TITLE-ABS-KEY("Granulocyte Colony-Stimulating Factor" OR (granulocyte AND colony AND stimulating AND factor) OR (gcsf) OR (neupogen) OR (filgrastim) OR (pegfilgrastim) OR (lenograstim) OR (molgramostim) OR (sargramostim))	99495
#4 #3 AND #2 AND #1	70

References

- [1] Vander Borgh M, Wyns C. Fertility and infertility: Definition and epidemiology. *Clinical Biochemistry*. 2018; 62: 2–10.
- [2] Chambers GM, Dyer S, Zegers-Hochschild F, de Mouzon J, Ishihara O, Banker M, *et al.* International Committee for Monitoring Assisted Reproductive Technologies world report: assisted reproductive technology, 2014†. *Human Reproduction*. 2021; 36: 2921–2934.
- [3] Chan JM, Sukumar AI, Ramalingam M, Ranbir Singh SS, Abdullah MF. The impact of endometrial thickness (EMT) on the day of human chorionic gonadotropin (hCG) administration on pregnancy outcomes: a 5-year retrospective cohort analysis in Malaysia. *Fertility Research and Practice*. 2018; 4: 5.
- [4] Jimenez PT, Schon SB, Odem RR, Ratts VS, Jungheim ES. A retrospective cross-sectional study: fresh cycle endometrial thickness is a sensitive predictor of inadequate endometrial thickness in frozen embryo transfer cycles. *Reproductive Biology and Endocrinology*. 2013; 11: 35.
- [5] Wu Y, Gao X, Lu X, Xi J, Jiang S, Sun Y, *et al.* Endometrial thickness affects the outcome of in vitro fertilization and embryo transfer in normal responders after GnRH antagonist administration. *Reproductive Biology and Endocrinology*. 2014; 12: 96.
- [6] Bu Z, Sun Y. The Impact of Endometrial Thickness on the Day of Human Chorionic Gonadotrophin (hCG) Administration on Ongoing Pregnancy Rate in Patients with Different Ovarian Response. *PLoS ONE*. 2015; 10: e0145703.
- [7] Aydin T, Kara M, Nurettin T. Relationship between Endometrial Thickness and In Vitro Fertilization-Intracytoplasmic Sperm Injection Outcome. *International Journal of Fertility & Sterility*. 2013; 7: 29–34.
- [8] Ranisavljevic N, Raad J, Anahory T, Grynberg M, Sonigo C. Embryo transfer strategy and therapeutic options in infertile patients with thin endometrium: a systematic review. *Journal of Assisted Reproduction and Genetics*. 2019; 36: 2217–2231.
- [9] Theyab A, Algahtani M, Alsharif KF, Hawsawi YM, Alghamdi A, Alghamdi A, *et al.* New insight into the mechanism of granulocyte colony-stimulating factor (G-CSF) that induces the mobilization of neutrophils. *Hematology*. 2021; 26: 628–636.
- [10] Ding J, Yang C, Zhang Y, Wang J, Zhang S, Guo D, *et al.* M2 macrophage-derived G-CSF promotes trophoblasts EMT, invasion and migration via activating PI3K/Akt/Erk1/2 pathway to mediate normal pregnancy. *Journal of Cellular and Molecular Medicine*. 2021; 25: 2136–2147.
- [11] Işık G, Oktem M, Guler I, Oktem E, Ozogul C, Saribas S, *et al.* The impact of granulocyte colony-stimulating factor (G-CSF) on thin endometrium of an animal model with rats. *Gynecological Endocrinology*. 2021; 37: 438–445.
- [12] Singh R, Singh M, Jindal A, Jindal PC. A prospective randomized controlled study (rct) of intra-uterine administration of granulocyte colony-stimulating factor (g-csf) before embryo-transfer on resistant thin endometrium in ivf cycles. *Human Reproduction*. 2015; 30: 280.
- [13] Sarvi F, Arabahmadi M, Alleyassin A, Aghahosseini M, Ghasemi M. Effect of Increased Endometrial Thickness and Implantation Rate by Granulocyte Colony-Stimulating Factor on Unresponsive Thin Endometrium in Fresh In Vitro Fertilization Cycles: A Randomized Clinical Trial. *Obstetrics and Gynecology International*. 2017; 2017: 3596079.
- [14] Singh R, Singh M. Rct of intra-uterine administration or subcutaneous injection of gcsf (granulocyte colony-stimulating factor) before embryo-transfer on resistant thin endometrium in ivf. *Human Reproduction*. 2018; 33: 276.
- [15] Eftekhari M, Sayadi M, Arabjahvani F. Transvaginal perfusion of G-CSF for infertile women with thin endometrium in frozen ET program: A non-randomized clinical trial. *Iranian Journal of Reproductive Medicine*. 2014; 12: 661–666.
- [16] Kunicki M, Łukaszuk K, Liss J, Skowrońska P, Szczyptańska J. Granulocyte colony stimulating factor treatment of resistant thin endometrium in women with frozen-thawed blastocyst transfer. *Systems Biology in Reproductive Medicine*. 2017; 63: 49–57.
- [17] Higgins JPT, Green S (editors). *Cochrane handbook for systematic reviews of interventions version 5.1.0 (updated march 2011)*. Cochrane, 2011. Available at: <https://training.cochrane.org/handbook/archive/v5.1/> (Accessed: 1 December 2022).
- [18] Slim K, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (minors): development and validation of a new instrument. *ANZ Journal of Surgery*. 2003; 73: 712–716.
- [19] Review manager (revman) [computer program]. Version 5.3.0. Copenhagen: The nordic cochrane centre. The cochrane collaboration, 2014. Available at: <http://tech.cochrane.org/revman/about-revman-5> (Accessed: 1 December 2022).
- [20] Xu B, Zhang Q, Hao J, Xu D, Li Y. Two protocols to treat thin endometrium with granulocyte colony-stimulating factor during frozen embryo transfer cycles. *Reproductive Biomedicine Online*. 2015; 30: 349–358.
- [21] Jindal PC, Singh M. A comparative rct of intrauterine-gcsf versus subcutaneous-gcsf in thin endometrium in ivf-icsi cycles. *Human Reproduction*. 2021; 36: deab130.346.
- [22] Zhang Y, Chen X, Chen S, Wei C, Li B, Wang Z, *et al.* Intrauterine administration of G-CSF for promoting endometrial growth after hysteroscopic adhesiolysis: a randomized controlled trial. *Human Reproduction*. 2022; 37: 725–733.
- [23] Singh N, Bahadur A, Mittal S, Malhotra N, Bhatt A. Predictive value of endometrial thickness, pattern and sub-endometrial blood flows on the day of hCG by 2D doppler in in-vitro fertilization cycles: A prospective clinical study from a tertiary care unit. *Journal of Human Reproductive Sciences*. 2011; 4: 29–33.
- [24] Lebovitz O, Orvieto R. Treating patients with “thin” endometrium - an ongoing challenge. *Gynecological Endocrinology*. 2014; 30: 409–414.
- [25] Brouard N, Driessen R, Short B, Simmons PJ. G-CSF increases mesenchymal precursor cell numbers in the bone marrow via an indirect mechanism involving osteoclast-mediated bone resorption. *Stem Cell Research*. 2010; 5: 65–75.
- [26] Xie Y, Tian Z, Qi Q, Li Z, Bi Y, Qin A, *et al.* The therapeutic effects and underlying mechanisms of the intrauterine perfusion of granulocyte colony-stimulating factor on a thin-endometrium rat model. *Life Sciences*. 2020; 260: 118439.
- [27] Zhao J, Tian T, Zhang Q, Wang Y, Li Y. Use of granulocyte colony-stimulating factor for the treatment of thin endometrium in experimental rats. *PLoS ONE*. 2013; 8: e82375.
- [28] Lyu M, Su A, Zhang L, Gao W, Liu K, Yue F, *et al.* Recombinant human granulocyte colony stimulating factor (rhG-CSF) participates in the progression of implantation via the hsa_circ_0001550-miRNA-mRNA interaction network. *Human Fertility*. 2022. (online ahead of print)
- [29] Rahmati M, Petitbarat M, Dubanchet S, Bensussan A, Chaouat G, Ledee N. Granulocyte-Colony Stimulating Factor related pathways tested on an endometrial ex-vivo model. *PLoS ONE*. 2014; 9: e102286.
- [30] Gleicher N, Vidali A, Barad DH. Successful treatment of unresponsive thin endometrium. *Fertility and Sterility*. 2011; 95: 2123.e13–e17.
- [31] Makinoda S, Mikuni M, Furuta I, Okuyama K, Sagawa T, Fujimoto S. Serum concentration of endogenous G-CSF in women during the menstrual cycle and pregnancy. *European Journal of Clinical Investigation*. 1995; 25: 877–879.
- [32] Gleicher N, Kim A, Michaeli T, Lee HJ, Shohat-Tal A, Lazaroni E, *et al.* A pilot cohort study of granulocyte colony-stimulating factor in the treatment of unresponsive thin en-

- dometrium resistant to standard therapies. *Human Reproduction*. 2013; 28: 172–177.
- [33] Mishra VV, Choudhary S, Sharma U, Aggarwal R, Agarwal R, Gandhi K, *et al*. Effects of Granulocyte Colony-Stimulating Factor (G-CSF) on Persistent Thin Endometrium in Frozen Embryo Transfer (FET) Cycles. *Journal of Obstetrics and Gynaecology of India*. 2016; 66: 407–411.
 - [34] Swati S, Sankalp S, Ashraf CM. Granulocyte colony stimulating factor(g-csf) : Does it really improve the endometrial thickness in women with persistent thin endometrial thickness undergoing frozen endometrial transfer? *Human Reproduction*. 2015; 30: 265.
 - [35] Kim YY, Jung YH, Jo JD, Kim MH, Yoo YJ, Kim S. The effect of transvaginal endometrial perfusion with granulocyte colony-stimulating factor (g-csf). *Fertility and Sterility*. 2012; 98: S183.
 - [36] Zheng Y, Chen B, Dai J, Xu B, Ai J, Jin L, *et al*. Thin endometrium is associated with higher risks of preterm birth and low birth weight after frozen single blastocyst transfer. *Frontiers in Endocrinology*. 2022; 13: 1040140.
 - [37] Kamath MS, Chittawar PB, Kirubakaran R, Mascarenhas M. Use of granulocyte-colony stimulating factor in assisted reproductive technology: A systematic review and meta-analysis. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*. 2017; 214: 16–24.
 - [38] Zhao J, Xu B, Xie S, Zhang Q, Li YP. Whether G-CSF administration has beneficial effect on the outcome after assisted reproductive technology? A systematic review and meta-analysis. *Reproductive Biology and Endocrinology*. 2016; 14: 62.