

REVIEW ARTICLE

Granulocyte Colony Stimulating Factor for Treatment of Thin Endometrium in Assisted Reproduction Technology Cycles

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ABSTRACT

Granulocyte colony stimulating factor (G-CSF), a glycoprotein, belongs to colony stimulating factor family and mainly regulates the growth and differentiation of granulocytes. However, it also plays an important role in endometrial stromal cell decidualization, ovulation, implantation, placental metabolism, trophoblast development and endometrial regeneration. It is due to these effects, it has been used in difficult clinical scenarios, such as unresponsive thin endometrium during assisted reproductive technology treatment, repeated implantation failure and recurrent miscarriages. Most of the studies have investigated its use in thin endometrium. In this review, we have summarized the current updated evidence with regards to use of G-CSF in women with thin endometrium.

Keywords: Assisted reproductive technology, Granulocyte colony stimulating factor, Thin endometrium.

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INTRODUCTION

The advent of assisted reproductive technology (ART) has helped millions of subfertile couples in achieving parenthood. In many ways, a positive ART cycle outcome is heralded by successful implantation of the transferred embryos. A successful implantation of an embryo in the uterus is the final outcome of a carefully orchestrated

sequence of events. Among many variables responsible for ART outcome, such as age of women, oocyte quality, sperm quality, embryo grades, and the endometrium, the least amount of attention has been given toward the endometrial receptivity.^{1,2}

The presence of pinopodes in the endometrial samples as observed through electron microscopy has been suggested as a marker of window of implantation.³ However in clinical practice, most often the ultrasound parameters, such as endometrial thickness, pattern, endometrial cavity volume and subendometrial Doppler flow are used to assess the endometrial receptivity.⁴ Among the ultrasound parameters, the endometrial thickness is more commonly used in ART practice. Various studies have suggested a minimum endometrial thickness between 6 and 8 mm for a successful ART outcome.⁵

The cut off for thin endometrium has been controversial, though many studies including donor recipient designs suggest a endometrial thickness of less 8 mm is associated with lower success.^{6,7} The thin endometrium is associated with impaired growth of glandular epithelium, reduced vascular endothelial growth factor, reduced subendometrial blood flow and high impedance of blood flow in the radial arteries.⁶

The probable causes for thin endometrium are—endometrial resistance to estrogen, impaired subendometrial blood flow, damage to basal endometrium following vigorous curettage and Asherman syndrome.⁴ Thin endometrium is a difficult condition encountered during ART cycle and often unresponsive to presently available treatment options.⁵ Some of the commonly used options are—oral and vaginal estrogen, aspirin, sildenafil citrate and sometimes as a last resort, surrogacy.⁵

It has been suggested that the growth factors, hormones and cytokines produced by the decidual cells, could probably play an important role in embryo implantation.⁸ Early studies have indicated granulocyte colony stimulating factor (G-CSF) has an important role in activating the macrophages and lymphocytes and suppressing immune response temporarily in the endometrium to facilitate embryo implantation.^{9,10}

The various immunological factors produced in the decidua are regulated locally resulting in a balanced

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immunoregulation of various immune cells, such as T-helper cells (T helper-1 and 2). The T helper-2 cells play important role in blocking the maternal T helper-1 cells which promote allograft rejection and in the process helping in continuation of pregnancy.¹¹ Granulocyte colony stimulating factor has been shown to help in recruiting dendritic cells, increase secretion of T helper-2 cells and promote angiogenesis.¹²

The suboptimal hormonal support due to endocrine ovarian pathology affects the endometrial immunoregulation manifesting in unresponsive endometrium.¹³

Granulocyte Colony Stimulating Factor: The Molecule

Granulocyte colony stimulating factor is a hormone like glycoprotein belonging to colony stimulating factor family regulating the hematopoietic cell growth and differentiation with G-CSF mainly stimulating the granulocytes colony formation. It is a 177 amino acid polypeptide with affinity for c-fms receptor which is present on the trophoblast surface.¹⁴ The trophoblastic cells express G-CSF receptors and it has been suggested that lack of expression of these receptors are linked to early miscarriage.^{15,16} The G-CSF has been found to beneficial effect on placental metabolism and trophoblast development.¹⁷

Numerous cells involved in the reproductive physiological function, such as endothelium, fibroblasts, monocytes and endometrial cells produce G-CSF. It also plays an important role in endometrial stromal cells decidualization.¹⁸

It has been suggested that G-CSF plays an important role during ovulation. It is involved in development and differentiation of luteinized granulosa cells.¹⁹ Its presence in the follicular fluid has been found to be a useful noninvasive marker of oocyte competency during ART.²⁰

The recombinant version of G-CSF was introduced three decades back for treating hematological conditions, such as neutropenia following chemotherapy, agranulocytosis, etc.^{21,22} Its promising role has been explored in reproductive medicine in cases of unresponsive thin endometrium, unexplained recurrent embryo failure and unexplained recurrent miscarriage mainly due to its beneficial effect on endometrial growth.^{10,23,24}

How does Recombinant G-CSF Work on the Endometrium?

The basic research has suggested that the G-CSF may be helped in stem cell mobilization, migration and ultimately differentiation which could lead to endometrial regeneration.²⁵ Due to its inhibitory effect on apoptotic activity, it may reduce endothelial cells death and promote

angiogenesis leading to increased endometrial vascularization.²⁶ The beneficial effect of G-CSF on endometrial receptivity leading to better implantations rates are further supported by higher success rates achieved in ART cycles following local endometrial injury, which leads to inflammatory reaction resulting in release of various growth factors and cytokines, such as G-CSF.²⁷

Recombinant G-CSF for Treatment of Thin Endometrium in ART: Current Evidence

Earlier study in a rat model, which looked at effect of G-CSF in thin endometrium, found histological evidence of more glandular growth and vascularization resulting in a thicker endometrium following administration of subcutaneous G-CSF compared saline, which was the control. The investigators suggested possible beneficial effect of G-CSF in promoting endometrial regeneration.²⁵

In their preliminary experience, Gleicher et al reported four ART cases, which included women with endometrial thickness of less than 7 mm, which did not respond to standard medical therapy. All four women conceived following transvaginal instillation of G-CSF in the uterine cavity, though one pregnancy was an interstitial ectopic pregnancy²⁸ (Table 1).

In their subsequent pilot cohort study, the investigators evaluated 21 subfertile women with thin endometrium observed on the day of trigger during ART cycle. Using an intrauterine catheter, approximately 30 mU of G-CSF was instilled in the endometrial cavity on the trigger day and reinstalled after 48 hours post-retrieval in case the endometrial thickness was still less than 7 mm. The endometrial thickness showed improvement from 6.4 ± 1.4 to 9.3 ± 2.1 mm by the transfer day which was significant. Ongoing pregnancy rate of 19.1% was obtained. The main study limitation was the small sample size and lack of control group. The study was supportive of role of G-CSF in treating chronic unresponsive endometrium during ART.¹⁰ In a similar prospective study, Kunicki et al evaluated role of G-CSF in 37 women with thin endometrium on the day of trigger.²⁹ All the women had at least one previously unsuccessful *in vitro* fertilization (IVF) with suboptimal endometrial thickness. The investigators found significant increase in endometrial thickness following G-CSF and pregnancy rate of 18.9%. However, the study was also limited by small sample. Similar nonrandomized study by Tehraninejad et al, involving 15 women with previously called ART due thin endometrium evaluated G-CSF on the day of oocyte retrieval. The author found some benefit of G-CSF in terms of increased endometrial thickness in this group of patients.³⁰



Table 1: Studies involving recombinant granulocyte colony stimulating factor in ART

S. No.	Author and year	Study design	Patient population	Intervention	Fresh or frozen embryo transfer	Endometrial thickness (ET)	Implantation / pregnancy rate	Remarks
1.	Gleicher et al (2011)	Case series	4 patients with at least one IVF failure undergoing egg donation cycle or frozen embryo transfer cycle	Intrauterine infusion of 30 MU (300 mcg/1 ml) r G-CSF performed minimum 48 hours before embryo transfer.	Egg donation/ frozen embryo transfer	Increase in ET >7 mm in all cases	100% (One was an ectopic pregnancy)	Small case series
2.	Kim YY et al (2012)	Prospective nonrandomized study	62 patients with poor endometrial growth divided into two groups Grp A (n 34) with poor endometrium and Grp B (n 28) with intrauterine synechia	Intrauterine infusion of 30 MU (300 cg/1 ml) r G-CSF performed on the day of hCG injection	Fresh cycle	Increase in ET ± 1.2 mm while no growth in Grp B	29.4% (Grp A) and 1.7% (Grp B) implantation rates	r G-CSF better in patients without intrauterine synechia
3.	Gleicher et al (2013)	Prospective nonrandomized study	21 patients with at least one IVF failure and endometrium < 7 mm on the day of hCG injection	Intrauterine infusion of 30 MU (300 cg/1 ml) r G-CSF performed on the day of hCG injection	Fresh cycle	Increase in ET ± 2 mm	Clinical pregnancy rates 19.1%	Cohort study small sample size
4.	Barad et al (2014)	Randomized double blinded placebo controlled trial	141 normal <i>in vitro</i> fertilization patients (73 r G-CSF group and 68 placebo group) with normal endometrium	Intrauterine infusion of 300 μ g/1 ml of r G-CSF on the day of hCG injection	Fresh	Similar increase in ET in both groups	No difference in implantation rates and pregnancy rates	Older women with normal thickness endometrium
5.	Li et al (2014)	Nonrandomized	59 patients undergoing frozen embryo transfer (34 r G-CSF group and 25 in control group)	Intrauterine infusion of 100 μ g/0.6 ml r G-CSF after 10 days of estrogen priming	Frozen	-	Similar pregnancy and implantation rates	Nonrandomized trial
6.	Kunicki et al (2014)	Nonrandomized	37 <i>in vitro</i> fertilization patients with endometrium < 7 mm on the day of hCG administration in previously failed ART cycle due to thin endometrium	Intrauterine infusion of 300 μ g/1 ml of r G-CSF on the day of hCG injection	Fresh	1.68 \pm 1.05 mm increase in ET	18.9% pregnancy rate	Small sample lack of control nonrandomized
7.	Shah et al (2014)	Prospective nonrandomized study	231 patients divided in Grp A (n-117) with thin endometrium less than 8 mm and Grp B (n-114) with implantation failures with endometrium more than 8 mm	Intrauterine infusion of 300 IU/ml r G-CSF performed after priming of endometrium for 10 days with estradiol valerate	Egg donation/ frozen embryo transfer	Mean increase in endometrium 2.5 mm after 4 days if infusion	37% in thin endometrium, 39.25% in implantation failure group	Nonrandomized lack of control
8.	Eftekhari et al (2014)	Prospective nonrandomized study	68 patients with thin ET < 7 mm divided into two groups	Intrauterine infusion on D 12-13th of COH, r G-CSF (300 μ g/1 ml) given to 34 patients in treatment group; no G-CSF in control	Frozen embryo transfer	No difference in ET in both groups	32.10 vs 12% nonsignificant difference in pregnancy rates	Nonrandomized
9.	Bin Xu et al (2015)	Prospective non randomized study	30 patients with ET < 7 mm and 52 control without intervention	Intervention arm divided into Grp A intrauterine infusion of 300 IU/ml r G-CSF performed after priming of endometrium for 10 days with estradiol valerate. Other Grp Endometrial scratch also performed	Frozen embryo transfer	Increase in 2.4 \pm 1.4 mm in ET with no difference in both intervention arm	Significantly higher clinical pregnancy rate 48.1% and implantation rate 31.5%	Endometrial scratch did not impair G-CSF treatment
10.	Mishra et al (2015)	Prospective study	35 patients with thin endometrium (<7 mm) resistant to standard treatments	Intrauterine infusion of GCSF (300 μ g/1 ml) was done in patients with thin endometrium on day 14 of frozen cycle	Frozen embryo transfer	Increase in ET >7 mm in 52% cases	Biochemical pregnancy rate of 15.1%; no clinical pregnancy	Small sample lack of control group
11.	Tehranejad et al (2015)	Nonrandomized trial	15 patients with chronic thin endometrium	Intrauterine infusion of 300 μ g/1 ml of r G-CSF on the day of ovum pickup	Fresh	Increase in ET by 3.5 \pm 0.88 mm	20% pregnancy rate	Nonrandomized small numbers lack of control

Barad et al, conducted a double blind placebo controlled trial to investigate the beneficial effect of G-CSF in women undergoing ART with normal endometrial thickness.³¹ A total of 73 women received G-CSF and 68 women were in the placebo group where saline was used. No difference was found in endometrial thickness after 5 days observation following G-CSF. The implantation (14.73 vs 15.98%) and pregnancy rates were also similar in both the groups. The study included older age group women (39.59 ± 5.56 years) with majority of patients having undergone at least one unsuccessful ART cycle (Table 1).

Kim et al, in their study included 62 women undergoing ART with thin endometrium and evaluated effectiveness of G-CSF in women with either intrauterine synechia or poor endometrial development.³² The study found beneficial effect of G-CSF in women who had poor endometrial development with significantly higher endometrial expansion being achieved (6.3 ± 1.4 to 8.7 ± 1.2 mm). However, it was not found to be effective in women with intrauterine synechia (6.1 ± 1.3 to 6.5 ± 1.4 mm) (Table 1).

In another prospective study, the role of G-CSF was evaluated in women with thin endometrium undergoing frozen embryo transfer (FET).³³ One group of women received G-CSF (300 µg/ml) which was administered through intrauterine insemination (IUI) catheter and control group did not receive it. A total of 68 women were evaluated and the cycle cancellation rate along with endometrial growth was similar in both the groups. Though the pregnancy rate was higher in G-CSF (32.10 vs 12%) compared to control group, this difference was not statistically significant. Overall the study did not show any benefit of G-CSF in FET cycles. A similar study evaluating G-CSF in patients with thin endometrium undergoing frozen embryo transfer did not find any benefit in terms of increased implantation or pregnancy rates.³⁴

In another prospective study by Mishra V et al, 35 women undergoing frozen embryo transfer who had thin endometrium (<7 mm) were recruited. All the women underwent intrauterine infusion of G-CSF (300 µg/1 ml) on the day 14 of the cycle and endometrium was measured 2 days later.³⁵ A total of 16 patients had cancellation of cycle due to inadequate endometrial thickness and three patients (15.78%) had biochemical pregnancies. No clinical pregnancies were obtained. The investigators did not find any increase in pregnancy rates following G-CSF instillation.

In a prospective study by Shah J et al, the investigators evaluated role of G-CSF in women with thin endometrium (n = 117) and those with repeated implantation failure but with a normal endometrium thickness (n = 114).³⁶

Women in both the groups underwent priming with estradiol valerate and sildenafil citrate for 10 days prior to infusion of G-CSF. Mean endometrial thickness increase of at least 2.5 mm was seen in 4 days following G-CSF and pregnancy rate of 37% in thin endometrium group along with 39.25% pregnancy rate in repeated implantation failure group was obtained. Lack of control group was one of the limitations of the study.

Xu et al evaluated two G-CSF protocols in women with thin endometrium undergoing frozen transfers. Out of 82 women who had previously canceled cycles, 30 received G-CSF.³⁷ This group was further subdivided into two groups—one received G-CSF only and second group underwent endometrial scratching along with G-CSF. The remaining 50 women served as a control. The endometrial thickness increase was significantly more in G-CSF group compared to previously canceled cycle. The subgroups which received G-CSF had similar thickness and pregnancy rates. However, significantly higher implantation and pregnancy rates were achieved in G-CSF group compared to control group and endometrial scratching did not affect the G-CSF treatment. Investigators suggested cancellation of fresh cycles with thin endometrium and subsequent frozen cycles with G-CSF pretreatment would be beneficial (Table 1).

Granulocyte colony stimulating factor has been used as therapeutic option in various conditions, such as patient undergoing bone marrow transplant, in treatment for neutropenia following chemotherapy, and in patients with neurodegenerative diseases.^{21,22} Some of the reported adverse effects include skin rashes, injection site rashes, bone pain, and myalgia. Most of these side effects have been reported in studies investigating role of G-CSF during hematological conditions.³⁸ No major side effects have been reported by studies looking at use of G-CSF in ART practice.

The G-CSF appears to be promising option for treatment of difficult conditions in ART practices, such as thin endometrium and repeated implantation failures. Most of the preliminary studies which suggested benefit were nonrandomized trials and a high quality randomized controlled trial did not show any benefit of G-CSF though it included women with normal endometrium. There is a need for larger properly designed randomized trial to ascertain the effectiveness of G-CSF in treating thin endometrium.

Summary Points

- Granulocyte colony stimulating factor, a glycoprotein, is involved in various reproductive functions, such as ovulation, decidualization, endometrial regeneration and receptivity.



- It also plays important role in facilitating trophoblasts development and placental metabolism, in the process, facilitating continuation of pregnancy.
- Early studies suggest beneficial role of G-CSF in patients with thin endometrium by increasing the endometrial thickness.
- Presently the supportive evidence consists of mainly observational studies. There is a need for larger well controlled randomized trials to establish the role of G-CSF in ART practice.

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