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Efficacy of subcutaneous granulocyte colony-stimulating factor infusion for treating thin endometrium

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Objective: This study was conducted to assess the efficacy of subcutaneous granulocyte colony-stimulating factor (G-CSF) for treating thin endometrium.
Methods: Data from 88 infertile women with thin endometrium (<7 mm) aged 23 to 40 years were evaluated retrospectively over a period of 1 year. In group 1, subcutaneous infusion of G-CSF (300 μg/mL) was administered to 44 women along with other supplemental treatments. If the lining did not exceed 7 mm within 72 hours, a second infusion was administered. In group 2, which also had 44 women, only estradiol valerate and sildenafil were administered, while subcutaneous G-CSF infusion was not. Embryo transfers were performed once the lining exceeded 7.5 mm. The efficacy of G-CSF was evaluated by assessing the thickness of the endometrium before embryo transfer, pregnancy rates, and clinical pregnancy rates.
Results: There were no differences between the groups regarding demographic variables, egg reserves, sperm parameters, the number of embryos transferred, and embryo quality. The pregnancy rate was significantly higher in group 1 (60%, 24 of 40 cases) than in group 2 (31%, 9 of 29 cases) (p<0.001). The clinical pregnancy rate was also significantly higher in group 1 (55%) than in group 2 (24%) (p<0.001).
Conclusion: Subcutaneous G-CSF infusion improved the thickness of the endometrium when it was thin. To the best of our knowledge, this is the first documented study to clearly demonstrate the benefits of subcutaneous G-CSF infusion for treating thin endometrium.

Keywords: Granulocyte colony-stimulating factor; Infusion; Subcutaneous; Thin endometrium

Introduction

Despite recent technological developments, *in vitro* fertilization (IVF) is still not a surefire solution to infertility, and implantation failure remains a major problem that leads to unsuccessful IVF. Successful implantation depends on many factors such as egg quality, sperm quality, embryo quality, the receptivity of the endometrium, and the quality of the embryo transfer technique [1]. The endometrium is very important for embryo implantation, and endometrial thickness is a marker of endometrial receptivity. It is used as a prognostic factor in embryo transfers [2,3]. Adequate endometrial thickness is essential for a successful pregnancy. A thin endometrium adversely affects the rate of successful reproduction using IVF. The definition of a thin endometrium varies across studies, but it is generally defined as an endometrium of < 7 mm on the day of human chorionic gonadotropin (hCG) injection during fresh embryo transfer cycles and < 7–8 mm on the day that progesterone supplementation is added for frozen-thawed cycles [4-6]. The reported prevalence of thin endometrium in assisted reproductive technology patients is between 1.5% and 9.1% [6,7].

There are various treatment regimens that aim to improve endometrial thickness, but only some have been found to yield actual improvements. Granulocyte colony-stimulating factor (G-CSF) has recently been suggested as a possible treatment for thin endometrium

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[8,9]. G-CSF is a glycoprotein that combines growth factor and cytokine activities. It is secreted in various tissues, including reproductive tissues such as the endothelium and ovarian follicles, as well as immunocytes (e.g., macrophages) [10]. It stimulates neutrophilic granulocyte proliferation and differentiation and acts on decidual macrophages, affecting implantation. It also recruits dendritic cells, promotes Th-2 cytokine secretion, activates T regulatory cells, and stimulates various proangiogenic effects that also affect implantation [11]. In most studies, intrauterine infusions of G-CSF were administered to improve the uterine lining [8,9]. However, G-CSF can also be administered subcutaneously to improve the thickness of the endometrium [12]. In this study, we examined the efficacy of subcutaneous G-CSF infusion for treating thin endometrium.

Methods

The study was conducted at the Advance Fertility and Gynaecology Centre in New Delhi, India, from January 2019 to December 2019. Patients with primary and secondary infertility between the ages of 23 to 40 years were included in the study. In total, 88 infertile women were examined, and the inclusion criteria were as follows. (1) A history of at least one previous IVF failure and thin endometrium (< 7 mm) 15 to 18 days into a regular 28-to-30-day cycle. (2) A history of canceled embryo transfer due to thin endometrium (< 7 mm) on the day of hCG injection. (3) A history of thin endometrium (< 7 mm) 12 or 13 days after beginning estrogen supplementation (6 to 10 mg/day).

In group 1, 44 women undergoing either fresh or frozen cycles were examined. Those undergoing fresh cycles received subcutaneous injections of G-CSF (300 μg/mL) starting on day 7 of hormonal injection along with oral estradiol valerate (4 to 6 mg/day) to increase endometrial thickness and vaginal sildenafil (50 mg/day) to increase uterine blood flow. Those undergoing frozen cycles received oral estradiol valerate (6 mg/day) starting on day 2 of the menstrual cycle and subcutaneous injections of G-CSF (300 μg/mL) starting on the 7th day after beginning medication, along with an increased dose of oral estradiol valerate (10 mg/day) and vaginal sildenafil (50 mg/day). If the endometrial thickness did not exceed 7.5 mm within 72 hours, a second injection was given, and the other supplements were continued. A final G-CSF injection was administered on the day of oocyte retrieval for those undergoing fresh cycles and on the day that progesterone was added for those undergoing frozen cycles.

In group 2, which also included 44 women, estradiol valerate and sildenafil were given to those undergoing fresh and frozen cycles like in group 1; however, subcutaneous G-CSF infusion was not administered (Figure 1). Embryo transfer was performed only when the endometrial thickness exceeded 7.5 mm and there was subjective improvement in the echotexture of the lining based on two-dimensional ultrasonography. The efficacy of G-CSF was evaluated by assessing endometrium thickness before embryo transfer, the pregnancy rate, and the clinical pregnancy rate.

Results

There were no significant differences between the groups regarding demographic variables, ovarian reserve (donor eggs were used in instances of low ovarian reserve), sperm parameters, number of embryos transferred, and embryo quality (Table 1).

In group 1, the embryo transfer was cancelled in four cases, and in group 2, there were 15 cancellations due to thin endometrium even after treatment, showing statistical significance ($p = 0.008$). The pregnancy rate was significantly higher in group 1 (60%, 24 out of 40 cases) than in group 2 (31%, 9 out of 29 cases) ($p < 0.001$). The clinical pregnancy rate was also significantly higher in group 1 (55%) than in group 2 (24%) ($p < 0.001$) (Table 2).

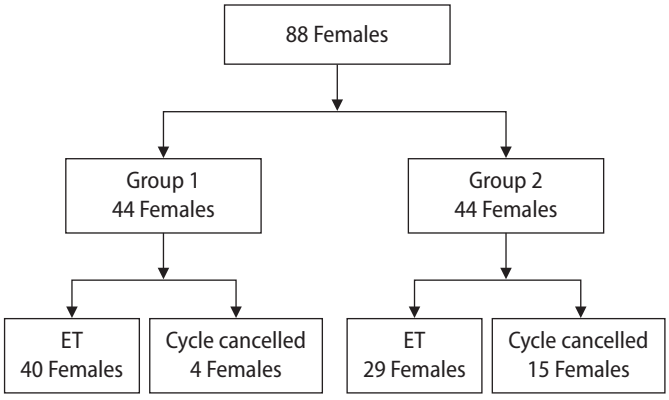

Figure 1. Flow diagram of study design. ET, embryo transfer.

Table 1. Demographic profile

Parameter	Group 1 (with G-CSF)	Group 2 (without G-CSF)	*p*-value
Number of patients	44	44	
Age (yr)	31.6	31.8	0.12
Length of infertility (yr)	4.3	5	0.47
Primary	25	23	1.12
Secondary	19	21	1.12
BMI (kg/m^2)	20.8	21	0.22
Antral follicle count	8.4	8.1	0.47
AMH level (ng/mL)	2.7	2.8	0.17

G-CSF, granulocyte colony-stimulating factor; BMI, body mass index; AMH, anti-Müllerian hormone.

Table 2. IVF outcome

Parameter	Group 1 (with G-CSF, n = 44)	Group 2 (without G-CSF, n = 44)	*p*-value
ET > 7.5 mm	40	29	< 0.001
Cycle cancelled	4	15	< 0.001
Pregnancy rate	24/40 (60)	9/29 (31)	< 0.001
Clinical pregnancy rate	22/40 (55)	7/29 (24)	< 0.001

Values are presented as number or number (%).
IVF, *in vitro* fertilization; G-CSF, granulocyte colony-stimulating factor; ET, endometrial thickness.

Discussion

Endometrial thickness is a marker of endometrial receptivity and is important for embryo implantation. Various treatments have been suggested in studies for improving endometrial thickness, but some remain unproven due to the limited number of subjects. In cases of thin endometrium, embryos from that particular cycle are frozen, and the endometrium is prepared again in order to perform an optimal embryo transfer.

G-CSF is an emerging treatment method for thin endometrium that has shown promising results. In our study, we assessed the effect of subcutaneous G-CSF infusion for improving the thickness of the endometrium. Zhang et al. [13] conducted a meta-analysis of 10 randomized control studies involving 1,016 IVF embryo transfer cycles, found that treatment with G-CSF infusion improved clinical outcomes after embryo transfer when performed using both local and systematic infusion, especially in cases of repeated implantation failure, and concluded that further randomized control trials are needed to investigate the efficacy of G-CSF infusion for patients with thin endometrium [13]. Most previous studies assessed the intrauterine effects of G-CSF infusion on thin endometrium. Gleicher et al. [8] and Kunicki et al. [9] found that intrauterine G-CSF infusion was effective for treating chronically thin endometrium and that the thickness of the endometrium significantly increased after G-CSF infusion, though it did not vary between conception and non-conception cycles. Both studies measured an ongoing clinical pregnancy rate of approximately 19%. Barad et al. [14] reported that G-CSF did not affect endometrial thickness, implantation rates, or clinical pregnancy rates among healthy IVF patients with normal endometrium or older IVF patients. Davari-Tanha et al. [15] conducted a double-blind placebo randomized control trial with 100 subjects in whom 300-μg intrauterine infusions of G-CSF were performed, and it was found that the infusions may have increased the chemical pregnancy and implantation rates of patients with recurring implantation failure; however, the clinical pregnancy rate and miscarriage rate were not affected. Very few studies have assessed the efficacy of subcutaneous G-CSF infusion for treating repeated implantation failure. Aleyasin et al. [12] found that single-dose systemic subcutaneous G-CSF infusion before implantation significantly increased the rates of successful IVF, implantation, and pregnancy (44.6%) in infertile women with repeated IVF failure. Scarpellini and Sbracia [16,17] found that G-CSF infusion might be effective for treating unexplained recurrent miscarriage and repeated implantation failure. Kamath et al. [18], in a Cochrane review, expressed uncertainty about the role of G-CSF for treating thin endometrium, and stated that the quality of the evidence suggesting that G-CSF infusion may improve the clinical pregnancy rate in women who have experienced two or more IVF failures was low. Zhao et al. [19] found that subcutaneous G-CSF infusion resulted in significantly higher pregnancy and implantation rates compared to the control group, whereas G-CSF administered via local uterine infusion had no beneficial effects on pregnancy and implantation rates in cases of recurrent IVF failure. Another recent study found that G-CSF infusion improved endometrial thickness regardless of whether the intrauterine or subcutaneous method was used. Although the intrauterine method showed slightly better results than the subcutaneous method, the degree of improvement was not statistically significant. Hence, the subcutaneous method can still be offered to patients, making it a viable option for performing G-CSF infusions to improve endometrial thickness and flow in patients with thin endometrium undergoing an embryo transfer [20].

Our study demonstrated the beneficial effects of G-CSF infusion using the subcutaneous method for treating thin endometrium, which is easier to administer than the intrauterine infusion method and does not require any extra steps. Subcutaneous G-CSF infusion was found to increase endometrial thickness and pregnancy rates in the study subjects. Other studies have examined the effects of subcutaneous G-CSF infusion in cases of recurrent IVF failure and unexplained recurrent miscarriages, but its effects on thin endometrium have not yet been established. Larger cohort studies are required in the future to further examine the effects of subcutaneous G-CSF infusion on thin endometrium. To the best of our knowledge, this is the first documented study to clearly demonstrate the beneficial effects of subcutaneous G-CSF infusion on thin endometrium.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Conceptualization: KB. Data curation: PV. Formal analysis: BS. Methodology: BS. Project administration: KB. Visualization: BS. Writing–original draft: BS. Writing–review & editing: BS.

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