Original Article

GCSF in patients with thin endometrium – subcutaneous or intrauterine?

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Abstract

Background: Granulocyte colony stimulating factor (GCSF) has a potential role in patients with persistently thin endometrium. Usually intrauterine route is employed for administering GCSF in patients with thin endometrium and data on subcutaneous route of administration is scarce. **Methods:** This was a randomized case control study from july 2018 to January 2019. Fifty patients with thin endometrium were enrolled in each group. In either group, GCSF was given if endometrium was less than 7mm on day 14, maximum of 2 doses. Primary outcome measured was increase in endometrium thickness and the secondary outcome was pregnancy rate. **Results:** Patients in both groups had similar endometrial thickness at the time of the initial evaluation: 5.27 mm in the subcutaneous and 5.34 mm in the intrauterine group. Similar change in the endometrial thickness was observed in the two groups: 1.76 in subcutaneous group and 1.84 in intrauterine group. It was observed that 61.2% had zone 3 blood flow in subcutaneous group compared to 74.1% in the intrauterine group, the difference being not statistically significant. Pregnancy rate of 40.1% in the subcutaneous group and 47.1% in intrauterine group was observed. ($P>0.50$). **Conclusion:** We concluded that G-CSF infusion leads to an improvement in endometrium thickness and this can achieved by both intrauterine and subcutaneous route. Intrauterine route is associated with slightly better results compared to subcutaneous route, though the improvement is not statistically significant. Hence, subcutaneous route can be offered to the patient, making it a viable option for administering GCSF to improve the endometrial thickness and flow in patients with thin endometrium undergoing embryo transfer.

Keywords: Embryo transfer, GCSF, subcutaneous

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INTRODUCTION

In vitro fertilization (IVF), through assisted reproductive technology (ART) is used to treat infertility. However, the success rate of IVF is still less than 40% with immense physical, emotional and financial burden on the couple[1] IVF success primarily relates to developing a good quality embryo and preparing a receptive endometrium.

The endometrial profile and endometrial preparation technique are two major determinants in women undergoing an embryo transfer procedure. Endometrial thickness (ET) is an important component in endometrial

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profiling and a clinical marker of endometrial receptivity.[2,3] Another important component is endometrial blood flow. Both good endometrial thickness and rich blood supply to the endometrium together predict robust implantation of embryo(s).

An endometrial thickness between 7 mm and 14 mm is considered optimum, which usually occurs about day 21 of the menstrual cycle.[4] Evidence-based data suggest decreased probability achieving of a pregnancy when the ET is 7 mm or less. In fact, an ET less than 7mm has negative effect on pregnancy.[5] Hence, thin endometrium is commonly defined as an endometrium thickness <7mm on the day of trigger or LH surge. Thin endometrium has been reported in 1-2.5% of the patients during standard IVF treatment.[6]

When despite the maximal dose of estrogen, an endometrium of desired thickness is not achieved, the physician can use other drugs which act by increasing the basal blood flow such as sildenafil, aspirin, pentoxifylline and tocopherol-f. A few cases however remain persistently thin despite all the above intervention. Such persistently thin endometrium cases are difficult to manage. Granulocyte colony-stimulating factor (GCSF) has a potential role in such cases. Recent studies have indicated a beneficial effect of GCSF in women who have thin endometrium, otherwise resistant to treatment.[7,8] A sudden increase in the thickness of the endometrium can often be noted within 48-72 hours of administration of GCSF.

GCSF, a glycoprotein, is a member of the colony-stimulating factor family of cytokines and growth factors. GCSF receptors have been found to be expressed in high concentration on dominant follicle, maximum being at preovulatory stage. Endometrium along with the luteinized granulosa cells also show an increased expression of these receptors at the time of ovulation till the time of implantation.[9] GCSF concentration rises in the follicular fluid at the same time. GCSF is also simultaneously found to be increased in significant proportions in serum during the ovulatory phase.[10] In a study by Salmassi *et al.*,[11] serum levels of GCSF was in direct correlation with levels of GCSF in follicular fluid. Serum levels increases progressively from the day the embryo is transferred to the day of embryo implantation and further increases once the pregnancy is confirmed and beyond as the period of gestation advances. This characteristic GCSF serum level curve is suggestive of significant function of GCSF in the process of implantation.

Changes in the endometrial thickness mediated by GCSF during implantation are immunologically mediated. GCSF also assists in the process of implantation by bringing about decidualisation of the endometrial stromal cells, in a cAMP-mediated process.[11] G-CSF also stimulates various endogenous endocrine mechanism, such as the secretion of endogenous cytokines which act both through the autocrine as well as the paracrine route.[10] Therefore, it is effective by both local and systemic routes when given from outside.

GCSF is safely used in the treatment of neutropenia during cancer chemotherapy, and no embryotoxic effects of this substance have been reported.[12] GCSF has no effect on embryonic chromosomal constitution.[13,14]

In a pilot study by Gleicher *et al.*,[7] four patients with unresponsive endometrium undergoing FET(frozen embryo transfer) were infused with GCSF into the uterus and all these patients conceived after infusion. Subsequently, the same authors described 21 infertile women with inadequate thin endometrium infused with GCSF and an ongoing clinical pregnancy rate of 19.2% was observed. The findings of Gleicher *et al.*[8] provided initial evidence that GCSF administration is beneficial in the treatment of infertile women with thin unresponsive endometrium.

Mishra *et al.*[15] conducted a similar study in patients undergoing FET cycles, and found a small increase in endometrial thickness after GCSF infusion but study failed to demonstrate any beneficial effect of GCSF in clinical pregnancy rate.[16]

GCSF was found to be beneficial in patients with thin endometrium and recurrent implantation failure. They observed that GCSF was efficacious when administered subcutaneously, resulting in significantly higher implantation rate and pregnancy rate. However, when administered locally by intrauterine infusion, GCSF showed no improvement in implantation and pregnancy rate.[17]

Even though we have robust evidence of efficacy of GCSF in increasing endometrial thickness, what is not clear is the appropriate route of administration. There is no available study comparing the efficacy of systemic and local route. Hence, we need to expand our knowledge in this regard. We intend to study the efficacy of the two routes in relation to endometrial thickness. We would also study the effect of GCSF on endometrial thickness and blood flow in patients

with unresponsive thin endometrium in women undergoing embryo transfer cycles.

MATERIALS AND METHOD

This randomized case control clinical trial was conducted at Institute of Reproductive Medicine and IVF center, Primus super specialty hospital for a period of 6 months from August 2018 to January 2019. 100 infertile women undergoing ART were included assuming proportion of thin endometrium(ET<7mm) patients to be 1%, absolute precision as 0.5% and level of confidence interval as 95%. The study was approved by ethical committee of the Indian fertility society.

Patients undergoing frozen embryo transfer were recruited in the study, after meeting the inclusion and exclusion criteria.

Inclusion criteria

(1) Primary and secondary infertility
(2) Age 18–45years
(3) Previous cycle cancellations because of thin unresponsive endometrium in spite of treatment.
(4) All patients undergoing embryo transfer with Inadequate endometrial lining response/thin endometrium (endometrial thickness less than 7 mm) in spite of receiving one or more adjuvant to improve endometrial lining.

Exclusion criteria

(1) Acquired uterine anomaly (polyp, submucosal myoma, Intrauterine adhesion, repeated dnc tuberculosis of the endometrium)
(2) Contraindication for GCSF (Presence of systemic diseases, endocrine disorders, renal disease, sickle cell disease, malignancy, pneumonia, chronic neutropenia)
(3) Women receiving infertility treatment for the first time
(4) Recurrent implantation failure
(5) Unwilling patients

Baseline TVS was performed on day 2 of cycle to assess baseline endometrial thickness and to rule out any uterine abnormalities. Patient was started on estradiol valerate (in titrating dose maximum of 12 mg/day − Tablet Progynova), low-dose aspirin, and vaginal sildenafil if required, was administered for endometrial preparation for 10 to 14 days. Endometrial thickness, pattern, and vascularity were assessed by TVS on day 12 and 14. If on day 14 endometrial thickness was less than 7 mm it was taken as thin endometrium.

Patients with thin endometrium were randomly allocated to one of the two groups using computer-generated random number of tables, into two groups:

Group A: Inj. GCSF (300 mcg/1 ml) subcutaneously on Day 14 onwards alternate days for two doses.

Group B: Inj. GCSF (300 mcg/1 ml) instilled slowly into the uterine cavity using an intrauterine insemination (IUI) catheter under USG guidance. Endometrial thickness was assessed after 48 h. If endometrial thickness was found to be <7 mm, a second infusion of GCSF was performed.

Injectable Progesterone 50mg intramuscular was started and embryo transfer was performed after 5 days. Luteal phase support was in form of daily intravenous injections of 50 mg progesterone for hormone replacement therapy cycles. Starting on the embryo-transfer day, 200-mg progesterone soft capsules were prescribed orally twice a day. Implantation was assessed using Serum β HCG test 15 days after embryo transfer. Pregnancy was confirmed by ultrasound documentation of gestational sac.

Ultrasound assessment of endometrial thickness and blood flow before and after GCSF was done. All the scan were performed by the same operator, using a Siemens Allegra machine, and a 7.5MHz transvaginal probe. Endometrial thickness is defined as the maximal distance between the echogenic interfaces of the endometrium and the myometrium in the plane of the central longitudinal axis of the uterus.

Primary outcome was measured in terms of endometrial thickness and endometrial blood flow and secondary outcome measured was pregnancy rate.

All patients were informed of their endometrial condition, present application status of GCSF, possible risks (e.g.

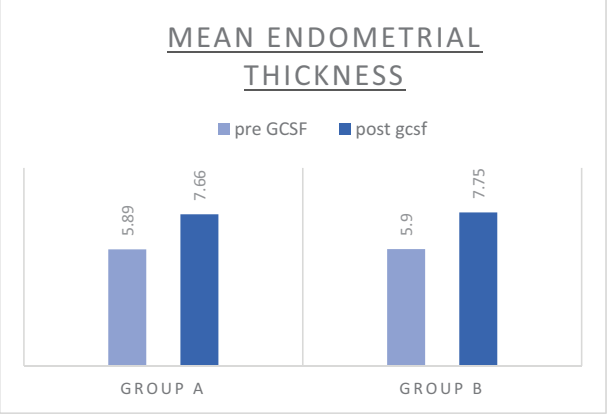

Figure 1: Mean endometrial thickness

Table 1: Baseline characteristics

Characteristics	Subcutaneous group	Intrauterine group
Primary infertility	60%	74%
Secondary infertility	40%	26%
Regular cycles	85.7%	94%
Mean cycle length(in days)	29.81	30.26
Mean age (in years)	33.28	32.92

Table 2: Comparison of etiology in relation to study groups

	Subcutaneous		Intrauterine	
	No.	%	No.	%
Tubal	9	18	7	14
Male	7	14	10	20
Ovarian	14	28	12	24
Combined	4	8	4	8
Unexplained	16	32	17	34
Total	50	100	50	100

fever, nausea headache, weakness, rash, sore muscles, interstitial pneumonia or shock), uncertain efficacy, and non-indicated use of GCSF and informed consent was taken before enrollment in the study.

STATISTICAL ANALYSIS PLAN

The data obtained were analysed using SPSS version 20.0. Continuous variables were expressed as mean ± SD values. Statistical tests such as unpaired t test were used to find significance of mean difference between two groups, chi square test was used to assess the relation between independent categorical variables. Probability value (*P* value) was used to determine the level of significance *P* value < 0.05 was considered as significant.

RESULTS AND OBSERVATIONS

100 patients were enrolled in each the study and were randomly allocated to group A (subcutaneous GCSF) and group B (intrauterine GCSF). All the patients were given two doses of GCSF. Patients who did not achieve an adequate endometrial thickness after 2 doses of GCSF (greater than 7mm) embryo transfer was cancelled and patients were given tab primolut-N to induce withdrawl bleeding. In group A two cycles were cancelled and three cycles in group B.

The mean age of the participants was 33.3 ± 5.12 years. Baseline characterstics of patients are given in Table 1. Distribution of patients according to the cause of infertility is shown in Table 2.

Endometrial thickness was measured on day 14 and after 2 doses of GCSF, results are shown in Figure 1. Figure 1 shows endometrial thickness in women before and after infusion of GCSF. In the subcutaneous group, the mean endometrial thickness before GCSF infusion was 5.89 ± 0.48 mm and, after infusion it increased to 7.66 ± 0.61 mm. Similarly, in the intrauterine group, the mean endometrial thickness before GCSF was 5.9 ± 0.53 which increased to a mean of 7.75 ± 0.43 after GCSF instillation. The Δ difference between endometrial thickness before and after intrauterine infusion of GCSF was 1.76 ± 0.6 in group A and 1.84 ± 0.6 in group B.

In the present study it was observed that after GCSF administration 61.2% had zone 3 blood flow in group A compared to 74.1% in group B, 36.7% had zone 2 blood flow in group A compared to 40.8% in group A. There was no statistically significant difference in between groups ($P > 0.05$).

In group A 20 patients conceived out of 48 patients (pregnancy rate 40.8%) and in group B 24 concieved out of 47 patients in whom GCSFwas instilled intrauterine (pregnancy rate 47.1%). This difference is small and not statistically significant.

DISCUSSION

We evaluated the efficacy of the two modes of GCSF administration, subcutaneous and intrauterine in patients undergoing embryo transfer with thin endometrium. The endometrium thickness increased significantly for the women in both the group (1.76 in subcutaneous group and 1.84 in intrauterine group). The change from pre to post Gcsf administration was similar in both the groups. Also, clinical pregnancy rates (subcutaneous: 40.8% and intrauterine: 47.1%) was similar in both groups.

GCSF can be administered by the subcutaneous as well as intrauterine route but which route is superior was a question still unanswered. Majority of the studies pertain to intrauterine instillation and data on subcutaneous administration is scarce. Studies done in relation to subcutaneous administration are limited to patient with recurrent implantation failure and not in patients with thin endometrium. Various studies have shown that intrauterine administration of GCSFin patients with thin and unresponsive endometrium causes increase in endometrial thickness.

Gleicher *et al.*[8] were the first to show the promising role of GCSF on endometrium expansion in women with

unresponsive endometrium in women undergoing FET. Since then many studies showed the positive influence of GCSF infusion in such patients, however the change in ET post GCSF is variable (1.5 to 3.5mm). In a recent metaanalysis by Xie *et al.*,[18] it was concluded that compared with control group, GCSF perfusion could significantly improve endometrial thickness with a mean difference of 1.79, 95% confidence interval (CI): 0.92-2.67. The results of this study were similar to the results observed in our study. We administered a dose of 300mg/ml of GCSF on day 14 and a repeat dose if required after 48 hours, was given if endometrial thickness did not reach a minimum of 7mm.

A recent study done on 30 patients used the same protocol, and found that endometrial thickness increased from 5.7 ± 0.7 mm to 8.1 ± 2.1 mm after GCSF treatment ($P < 0.001$) We found a similar increase in ET(5.9 ± 0.53 mm to 7.75 ± 0.43mm) in our study, in patients who were given intrauterine instillation of GCSF. Thus, reemphasizing the vital role of GCSF in patients with thin endometrium.[18]

We also found an improvement in the subendometrial blood flow with the use of intrauterine gcsf in patients with thin endometrium. 60% of patients showed an improvement in blood flow to zone 3 and 30% had blood flow improved to zone 2. This is the first study to report improvement in subendometrial blood flow after GCSF administration.

Another important consideration in our study was whether the subcutaneous route offers similar results in patients with thin endometrium as compared to intrauterine instillation. Interestingly, the change in ET and blood flow was similar in both the groups, which means subcutaneous route can be a potential alternative to intrauterine instillation as intrauterine group is more cumbersome and painful. Ours is probably the first study from India to look at this comparison between the two routes. Even the data from western literature is scarce in this regard and the need for such studies has been highlighted in the literature.

One study has evaluated the effect of GCSF administration on pregnancy rate (PR) according to the route of GCSF administration. The results showed an increased PR when GCSF was administrated via subcutaneous injection (OR 3.12), and a similar PR when GCSF was given via uterine infusion (OR 1.43).[17] Their results was different from our study where we found slightly less pregnancy rate in patients who were given subcutaneous GCSF. This is attributed to possibly the fact that the metaanalysis had included studies on use of GCSF in patients with recurent implantation failure (RIF) also. Hence, the results from this metaanalysis cannot be compared with our study because the study population is not similar. In the present study, compared to intrauterine instillation, the results were similar in subcutaneous route as well in terms of change in endometrial thickness (1.76 ± 0.6 versus 1.84 ± 0.6), blood flow (61.2% versus 71.4%), and clinical pregnancy rate (40.8% versus 47.1%). Immunological mechanisms in the endometrium are involved in the implantation process. GCSF boosts the endogenous cytokines' secretion and enables various different endocrine routes. In a study by Tanaka and colleagues it has been postulated that, GCSF causes decidualization of endometrial stromal cells by both the autocrine and paracrine routes. Presence of GCSF receptors on decidual and trophoblast has been found, through which they aid in the process of implantation.[10] These reports are similar to our findings and support them in association with improving endometrial thickness and pregnancy rate similar to that seen by subcutaneous route.

Subcutaneous route offers many advantages. It is considered more convenient to the patient, causes less discomfort and pain, less time consuming. Although during the study it was observed that few patients who were given subcutaneous administration, might need extra one or more dose of GCSF for achieving similar results. Based on this it is speculated that in subcutaneous route, total number of injections required might be higher than intrauterine route. The appropriate dosing regime has not been formally evaluated and needs further research. Subcutaneous injections have a potential to cause skin reaction and myalgias, but none were reported, meaning that it is a safe technique.

Though our study was a randomized control trial which clearly showed that subcutaneous route is equally efficacious as compared to intrauterine instillation of GCSF in patients with thin endometrium undergoing embryo transfer, but there are few potential limitations. Small sample size because of time-bound period of the study. Confounders such as obesity, smoking and alcohol intake, presence of adenomyosis and endometriosis, were not taken into consideration, though prevalence of obesity is usually low in Indian women who are mostly malnourished. Also, habits of smoking and alcohol intake are exceedingly uncommon in Indian women compared to Western population.

As the patients were undergoing treatment for endometrial preparation for embryo transfer, patient was already on estrogen and low-dose aspirin and vaginal sildenafil, so we are not sure whether the increment seen in endometrial thickness was an effect of GCSF alone or it was due to a combined effect of all these preparations. The very fact that Gcsf was used after these early measures failed to make this possibility unlikely.

CONCLUSION

Use of GCSF plays an important role in management of patients of thin endometrium undergoing embryo transfer. Subcutaneous route of administration seems to be equally effective as intrauterine instillation with the advantage of ease of administration and comfort to the patient, making it more acceptable to the patient. Hence, it has the potential to become a more popular technique of GCSF administration in near future.

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Conflicts of interest
There are no conflicts of interest.

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