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ORIGINAL ARTICLE

Effects of Granulocyte Colony-Stimulating Factor (GCSF) on Persistent Thin Endometrium in Frozen Embryo Transfer (FET) Cycles

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Abstract

Objective To predict the effectiveness of granulocyte colony-stimulating factor (GCSF) in the treatment of persistent thin endometrium resistant to other treatments in frozen embryo transfer (FET) cycles.

Study Design This is a hospital-based prospective study.

Patients Thirty-five women with persistent thin endometrium (<7 mm) resistant to standard treatments were involved in this study.

Intervention(s) Intrauterine infusion of GCSF (300 mcg/1 ml) was done in patients with thin endometrium on day 14 of FET cycles, and their endometrial thicknesses were measured after 48 h of infusion.

Main Outcome Measures The primary outcome was an increase in endometrial thickness and the secondary outcome measures were chemical and clinical pregnancies.

Results The endometrial thickness increased from 5.86 ± 0.58 to 6.58 ± 0.84 mm after GCSF infusion. In 19 of the 35 participants (54.28 %) endometrial thickness increased to ≥7 mm and they subsequently underwent embryo transfer. Of these, 3 (15.78 %) patients had chemical pregnancy, but there was no clinical pregnancy.

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In 16 participants, embryo transfer was canceled in view of insufficient endometrial thickness (<7 mm).

Conclusion GCSF caused a small increase in endometrial thickness in women with persistent thin endometrium, but there was no improvement in their pregnancy rates.

Keywords GCSF · Frozen embryo transfer cycles · Thin endometrium · Chemical and clinical pregnancy

Introduction

Persistent thin endometrium resistant to standard treatments affects <1 % of patients undergoing in vitro fertilization (IVF) and is really a frustrating problem both for the treating physician as well as the patient. It remains a constant challenge leading to unnecessary cycle cancellation and treatment delay. Various treatments have been proposed, including extended estrogen administration, low-dose aspirin, pentoxifylline, tocopherol, and vaginal sildenafil citrate, but have found to be ineffective in such patients [1, 2].

Transvaginal sonography (TVS) is used to assess endometrial thickness and vascularity during IVF treatment. An endometrial thickness of >7 mm with good endometrial vascularity is usually considered to be an essential requirement for implantation. The role of endometrium in implantation has attracted a lot of attention in recent years.

It has now been well established that an endometrial thickness of <7 mm is suboptimal for embryo transfer and has a negative effect on pregnancy rate [3–5]. There are very few options for increasing the thickness of a persistently thin endometrium.

Recent reports have suggested that intrauterine perfusion with granulocyte colony-stimulating factor (GCSF) may be effective in women who are otherwise resistant to treatment.

Gleicher et al. have reported the successful use of GCSF in four women undergoing IVF cycles, in whom standard treatments to improve endometrial thickness had failed [6]. GCSF has been found to be useful in the treatment of repeated implantation failures and miscarriages [7, 8].

GCSF is a glycoprotein and possesses growth factor and cytokine functions. There are various immunological mechanisms involved in the implantation process. GCSF stimulates the secretion of various endogenous cytokines and also activates various endocrine pathways. In a study by Tanaka et al., it was concluded that GCSF acts both in an autocrine and a paracrine fashion and promotes decidualization of endometrial stromal cells [9].

Various studies have shown that GCSF acts on decidual cell macrophages and affects implantation by stimulating neutrophilic granulocyte proliferation and differentiation [10, 11]. GCSF causes dendritic cell recruitment and T-regulatory cell activation and stimulates various proangiogenic factors [12].

The aim of this study was to assess the effects of intrauterine instillation of GCSF on unresponsive thin (<7 mm) endometrium in women undergoing frozen embryo transfer (FET) cycles.

Materials and Methods

This was a prospective study conducted at the Obstetrics and Gynecology Department of Institute of Kidney Diseases and Research centre, Civil Hospital, Ahmedabad from Jan 2014 to Dec 2014 and was approved by the institutional ethical committee.

A total of 35 women with persistent thin endometrium (<7 mm) unresponsive to other treatments and undergoing frozen embryo transfer cycles were included in this study.

Inclusion criteria

1. Women aged 20–45 years.
2. Previous cycle cancellations because of thin unresponsive endometrium in spite of treatment.
3. Normal uterine cavity as confirmed by office hysteroscopy.

Exclusion criteria

1. Presence of systemic diseases, endocrine disorders, sickle cell disease, chronic neutropenia, and history of malignancy and renal insufficiency.
2. Presence of Asherman's syndrome, fibroids, and polyps on hysteroscopy.

Informed consent was obtained from all women included in this study. Baseline TVS was done using Voluson E 8 machine on day 2 of cycle and estradiol valerate, low-dose aspirin, and vaginal sildenafil were administered for endometrial preparation. Endometrial thickness, pattern, vascularity and flow indices were assessed by TVS on day 14. If endometrial thickness was <7 mm, GCSF (300 mcg/1 ml) was instilled slowly into the uterine cavity using an intrauterine insemination (IUI) catheter under USG guidance. Endometrial thickness was assessed after 48 h and decision was made accordingly. If endometrial thickness was <7 mm, either a second infusion of GCSF was performed or cycle was canceled, and if endometrial thickness was >7 mm, progesterone was started and embryo transfer was done on day 3.

The primary outcome measure was an increase in endometrial thickness and the secondary outcome

measures were chemical pregnancy (serum beta hCG titer >25 mIU/ml) and clinical pregnancy defined as the presence of an intrauterine gestational sac on TVS at 6 weeks.

Statistical Analysis

Statistical analysis was done using data analysis software system, Statistical Package for Social Sciences (SPSS Version 20). Continuous data were expressed as mean ± SD. Paired t test and Wilcoxon rank sum test were applied for the continuous data. $P < 0.05$ was considered statistically significant.

Results

The baseline characteristics of the participants are shown in Table 1. The mean age of the participants was 30.49 ± 6.89 years and the mean BMI was 23.81 ± 3.62 kg/m^2. Table 2 shows the baseline hormonal profile of the subjects. Distribution of patients according to the cause of infertility is shown in Table 3.

Table 4 shows endometrial thickness in women before and after infusion of GCSF. In all the subjects, the mean endometrial thickness before GCSF infusion was 5.86 ± 0.58 mm, and, after infusion, it increased to 6.58 ± 0.84 mm. Although the difference was 0.72 mm which was statistically significant, the thickness was still <7 mm which was unfavorable for embryo transfer. Endometrial thickness increased to ≥7 mm only in 19 of the 35 participants (54.28 %) who then underwent embryo transfer. Of these, 3 (15.78 %) patients had chemical pregnancy but none of them had clinical pregnancy. In 16 participants, embryo transfer was canceled in view of insufficient endometrial thickness (<7 mm) even after GCSF infusion. Nine of the 35 participants were given second infusion of GCSF.

The endometrial volume and vascular indices—vascularity index (VI), flow index (FI), vascularisation flow index (VFI)—were also assessed before and after GCSF infusion, but there was no statistically significant difference (Table 5). No side effects of GCSF were noted in our study.

Table 3 Distribution according to cause of infertility

Infertility diagnosis	Frequency	Percentage (%)
Ovarian	7/35	20
Male factor	8/35	22.85
Tubal factor	11/35	31.42
Unexplained	5/35	14.28
Combined	4/35	11.42

Discussion

Persistent thin endometrium (<7 mm) still remains an unresolved problem and presents a clinical dilemma for the treating physician.

Table 1 Baseline characteristics

S. no.	Characteristics	Total ($N = 35$)	Pregnant ($N = 3$)	Non-pregnant ($N = 16$)	P value
1	Mean Age (years)	30.49 ± 6.89	30.33 ± 8.74	32.06 ± 7.51	0.72 (NS)
2	Type Of Infertility				
	Primary	28	2	14	0.42 (NS)
	Secondary	7	1	2	
3	BMI (Kg/m^2)	23.81 ± 3.62	23.17 ± 4.25	24.05 ± 3.89	0.73 (NS)
4	Previous IVF attempts	0.37 ± 0.60	0	0.50 ± 0.73	–

Continuous variables are shown as mean ± standard deviation. $P < 0.05$ considered to be statistically significant. NS represents non-significant difference between these groups

Table 2 Hormonal characteristics

Variables	Total ($N = 35$)	Pregnant ($N = 3$)	Non-pregnant ($N = 16$)	P value
FSH (mIU/ml)	9.23 ± 7.77	7.07 ± 3.53	9.08 ± 5.62	0.56 (NS)
LH (mIU/ml)	6.43 ± 4.72	4.27 ± 0.32	6.05 ± 3.50	0.40 (NS)
TSH (μg/dl)	2.27 ± 1.05	2.13 ± 0.86	2.04 ± 0.99	0.89 (NS)
PRL (ng/ml)	11.78 ± 3.91	14.32 ± 3.41	9.97 ± 3.29	0.05 (NS)
Estradiol (pg/ml)	41.85 ± 37.46	34.38 ± 17.19	45.67 ± 10.59	0.14 (NS)

NS represents non-significant difference between these groups

$P < 0.05$ considered to be statistically significant

Table 4 Endometrial thickness (ET) before and after GCSF infusion

	ET before GCSF (mm)	ET after GCSF (mm)	Difference in ET (mm)	*P* value
All women ($N = 35$)	5.86 ± 0.58	6.58 ± 0.84	0.72	<0.01*
Embryo transfer ($N = 19$)	6.03 ± 0.53	7.19 ± 0.42	1.16	<0.01*

Data are shown as mean ± standard deviation

P value <0.05 considered to be statistically significant

* Statistically significant difference between these groups

Table 5 Endometrial vascular indices before & after GCSF infusion

Variables	Before GCSF	After GCSF	*P* value
Endometrial volume (mm^3)	1.77 ± 1.01	1.89 ± 0.96	0.25 (NS)
VI	2.83 ± 3.95	3.22 ± 4.05	0.53 (NS)
FI	22.80 ± 3.07	22.25 ± 2.99	0.74 (NS)
VFI	1.12 ± 1.62	1.83 ± 4.88	0.93 (NS)

NS represents non-significant difference between these groups

$P < 0.05$ considered to be statistically significant

In a study by Gleicher et al., four patients with unresponsive endometrium undergoing IVF treatment were infused with GCSF into the uterus and all these patients conceived after infusion [6]. Subsequently, the same authors described 21 infertile women with inadequate thin endometrium infused with GCSF and an ongoing clinical pregnancy rate was observed, 19.1 %. The findings of Gleicher et al. provided evidence that GCSF administration is beneficial in the treatment of infertile women with thin unresponsive endometrium [13].

In our study, the participants were on FET program unlike that in Gleicher et al. which involved fresh IVF stimulation cycles. In a study by Gleicher, GCSF was administered on the day of hCG administration and the first assessment of endometrium was done after 48 h. In our study, the first dose of GCSF was given on the 14th day of FET cycle and the endometrium was assessed after 48 h similar to that in Gleicher et al. but in contrast to that in Kunicki et al. where endometrial thickness was measured after 72 h of GCSF instillation [13, 14].

The dose of GCSF used for intrauterine infusion in our study was 300 mcg/1 ml similar to that in Gleicher et al. and Kunicki et al. and 9 of the 35 (25.7 %) participants received second GCSF infusion 48 h after the first dose. In a study by Gleicher et al., 3 out of 21 (14.35 %) patients received second infusion of GCSF. In contrast, Kunicki et al. used a single GCSF infusion in their study [13, 14].

The population in our study was younger in contrast to Gleicher et al. and Michal Kunicki et al. (30.4/40.5/34.6 years) [13, 14].

In our study, endometrial thickness increased from 5.8 mm to 6.5 mm after GCSF administration. Only in 19 out of 35 (54.28 %) women, endometrial thickness reached at least 7 mm and therefore they subsequently underwent embryo transfers on day 3. Cycle cancellation was done in 16 (45.7 %) patients in view of insufficient endometrial thickness even after GCSF administration. Out of 19 women who underwent embryo transfer, 3 (15.78 %) patients had chemical pregnancy, but none had clinical pregnancy. In contrast, in a study by Gleicher et al., the endometrial thickness increased from 6.4 to 9.3 mm, with a difference of 2.9 mm which was statistically significant. All 21 women underwent embryo transfer and there was an ongoing clinical pregnancy rate of 19.1 % [13].

In a study by Kunicki et al., 37 women underwent GCSF intrauterine infusion for thin endometrium, and it was found that the endometrium significantly increased after GCSF infusion and the clinical pregnancy rate was 18.9 % in contrast to our study in which there was no clinical pregnancy [14].

In a recent study by Eftekhar et al. on 68 infertile patients with thin endometrium undergoing FET cycles, 34 patients received intrauterine GCSF (300 mcg/1 ml) instillation on 12th–13th day of cycle followed by a second infusion after 48 h if the endometrium was still <7 mm. The cycle cancellation rate was 15.20 % similar to the control group. The endometrial growth was also similar in both the groups. In contrast, cycle cancellation was done in 16 (45.7 %) patients in our study. This study failed to demonstrate that GCSF improves endometrial thickness but has the potential to improve chemical and clinical pregnancies [15].

A recent study by Li et al. failed to demonstrate any beneficial effect of GCSF on implantation or clinical pregnancy rates in infertile women with thin endometrium in an FET program. They used 100 mcg dose of GCSF and only one instillation in their study [16].

Kim et al. found that endometrial perfusion with GCSF in women with thin endometrium and recurrent IVF failures helped in improving endometrial thickness in patients without synechiae with an increase in implantation rates and ongoing pregnancy rates, but there was no effect in patients with synechiae [17].

GCSF can be administered by the subcutaneous as well as intrauterine route but which route is superior remains to

be determined. In addition, studies are required to see whether multiple doses are superior to a single infusion. The limitations in our study were a small sample size and a lack of a control group. In our study, we found a small but significant increment in endometrial thickness after GCSF infusion. As the cycles were FET cycles, patient were already on estrogen and low-dose aspirin, so we are not sure whether the small increment seen in endometrial thickness was an effect of GCSF alone or it was due to a combined effect of all these preparations.

Conclusion

In our study, we found a small increase in endometrial thickness after GCSF infusion but our study failed to demonstrate any beneficial effect of GCSF on clinical pregnancy rates in patients with thin endometrium in FET cycles. Further large prospective studies are required to establish the role of this relatively new drug in the treatment of thin unresponsive endometrium.

Compliance with Ethical Standards

Conflict of interest Vineet Mishra, Sumesh Choudhary, Urmila Sharma, Rohina Aggarwal, Ritu Agarwal, Khushali Gandhi, and Nilesh Goraniya declare that they have no conflicts of interest.

Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

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