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Comparative Study of The Role of Single Versus Double Dose of GCSF in Poor Endometrial Thickness Before Frozen Embryo Transfer

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Abstract

Background: Endometrial thickness is one of the most crucial predictor of success in cases of in vitro fertilization. Many cycles eventually get cancelled due to poor endometrial thickness(ET). The role of granulocyte colony stimulating factor(G-CSF) in enhancing persistently thin endometrium has emerged in the literature over the last decade.

Objectives: To assess the effect of intrauterine G-CSF instillation on unresponsive thin endometrium and to compare the ET following single and double dose instillation.

Methods: This is a prospective study carried out on 150 patients recruited for frozen – thawed embryo transfer. All patients were started on oral oestradiol valerate 6 mg daily and topical oestradiol 5 mg twice daily from day 3 of menstrual cycle.ET was measured on day 12 of menstruation by transvaginal ultra-sonography. Patients who had ET 5.5 mm were randomized into two groups. Both the groups received first dose intrauterine instillation of 300 mcg G-CSF on day 12. ET was measured 72 hours after this. Group B subjects received a second dose of G-CSF and ET was again measured 72 hours later. Study end point of group A was 72 hours after the first dose and that of group B was 72 hours after second dose.

Results: Finally, 72 patients in group A and 61 patients in group B were available for analysis. In group A the mean ET was 6.46mm (SD ± 0.43) and in group B, it was 7.28mm (SD ± 0.50), the difference being statistically significant with p value of <0.001

Conclusions: Intrauterine G-CSF instillation results in significant increase of ET in cases of persistently thin endometrium. Double dose of G-CSF instillation leads to statistically significant increase of ET, compared to single dose.

Keywords: FET, Thin endometrium, G-CSF

Introduction

Endometrial thickness is an important predictor of implantation and subsequent pregnancy in in-vitro-fertilization(IVF). Generally, ET of 7mm or more is considered favorable for embryo transfer. Various pharmacological agents are used to increase ET. Unresponsive, thin endometrium remains a great challenge in the field of assisted reproduction and many cycles eventually get cancelled due to poor endometrial thickness.

Over the last decade, Granulocyte colony stimulating factor(G-CSF) has emerged as a promising option for enhancing endometrial thickness. G-CSF is a glycoprotein which stimulates granulocyte production in bone marrow. Traditionally, it has been used to treat chemotherapy induced neutropenia. It has found its use in reproductive medicine by virtue of its ability to improve ovarian function[1] in general and granulosa cell function[2]. It improves ovarian stimulation in poor responders[3] and also reduces unexplained repeated pregnancy loss[4].

Gleicher and colleages in 2011[5] first reported increase in ET and subsequent successful pregnancy following

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intrauterine G-CSF instillation in unresponsive thin endometrium[5]. Kunicki et al[6] also demonstrated increased ET after intrauterine G-CSF perfusion, but they did not find any improvement in clinical pregnancy rate.

Suboptimal ET after such treatment has also been described by some author[7].

We took up this study to evaluate the effect of a second dose of intrauterine G-CSF instillation on thin endometrium and also to compare the effect of single dose and double dose.

Aims and Objectives

1. To assess the effect of intrauterine GCSF instillation on unresponsive thin endometrium.
2. To compare the effect of single dose and double dose intrauterine GCSF instillation on thin endometrium.

Materials and Methods

A prospective observational cohort study was carried out in a private infertility center for one year period between January and December 2019. Study was conducted on 150 women (aged between 23 to 40 years), planned for frozen-thawed embryo transfer (FET).

Inclusion criteria: -

1. Two consecutive cancelled cycles
2. Thin endometrium (ET less than 5.5mm on day12 of menstruation after receiving oral and topical estrogen therapy

Exclusion criteria: -

1. Intrauterine adhesions
2. Adenomyoma
3. Intramural fibroids
4. Contraindication to G-CSF

150 consecutive patients meeting the inclusion criteria were selected for study and informed consent regarding participation in the study were obtained. All patients received oral estradiol valerate 6mg daily and topical estradiol gel 5gm twice daily from day 3 of menstrual cycle. Patients were randomized into two groups, odd numbers belonging to group A and even numbers constituting of group B. The patients were blind to which group they were assigned to. On day 12 endometrial thickness was measured in millimeter by transvaginal ultrasonography (GE Voluson E-8). The sonographic measurement was taken at the most expanded area of endometrial stripe. Ultrasonic examination was done by a single operator throughout the study.

Patients belonging to both groups then received 300mcg of G-CSF(Endokine, Filgrastin, Intas Pharmaceuticals, India) into the uterine cavity on day 12. The drug was delivered through intrauterine insemination (IUI) catheter, under ultrasound guidance in full bladder. All patients had their ET measured after 72 hours of G-CSF instillation. For group A this was the study end point. Patients in group B received a second dose of 300 mcg intrauterine instillation 72 hours after the first dose and ET was measured again after 72 hours, this being the study end point for this group. Complications or adverse effects of the treatment were noted.

Results were statistically analyzed using Fisher's Exact Test (P Value <0.001 - Significant).

Results

150 women (aged between 23 to 40 years), planned for frozen-thawed embryo transfer (FET) were taken up for study. Patients were initially equally divided into group A and B. 3 patients in group A did not turn up for follow up and therefore 72 patients in this group were available for analysis. In group B, 2 patients were lost to follow up. Another 12 patients were excluded from study since they already had ET of 7 or more after first dose of G-CSF. Therefore, we finally had 72 patients in group A and 61 patients in group B for analysis. No side effects were noted in our series.

In group A, out of 72 patients receiving single dose of G-CSF, majority women i.e. 37 (51.4%) had ET increase in the range of 6.5 to 6.9 mm in as depicted

in table 1. Twenty patients (27.8%) had ET increase in the range of 6 to 6.4 mm and 11 (15.28%) reached the desirable ET of 7 mm or more.

Table 1 – showing crosstabulation of Endometrial thickness (ET) in mm

		GROUP		
		GROUP A	GROUP B	Total
Endometrial thickness	5.5 to 5.9	4	2	6
		5.6%	3.3%	4.5%
	6 to 6.4	20	2	22
		27.8%	3.3%	16.5%
	6.5 to 6.9	37	10	47
		51.4%	16.4%	35.3%
	7 to 7.4	9	5	14
		12.5%	8.2%	10.5%
	7.5 to 7.9	2	39	41
		2.8%	63.9%	30.8%
	8 to 8.4	0	3	3
		0.0%	4.9%	2.3%
Total		72	61	133
		100.0%	100.0%	100.0%

In group B, out of 61 patients receiving double dose of G-CSF, majority women i.e. 39 (63.9 %) had ET increase in the range of 7.5 to 7.9 mm . Next in order are 10 patients (16.45) with ET of 6.5 to 6.9 mm and 5 (8.2%) with 7 to 7.4 mm. Three patients (4.9%) achieved ET of more than 8mm. (table 2)

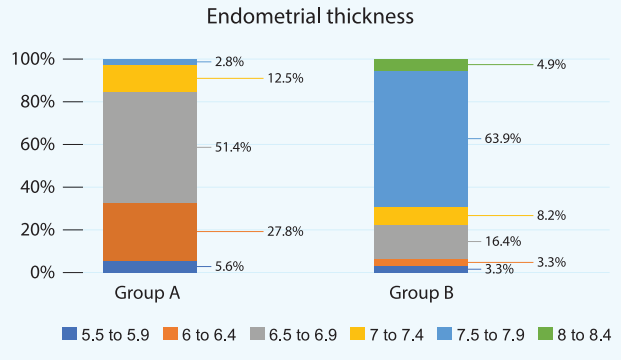

Table 2: Bar diagram showing range of endometrial thickness in mm

Mean increase of ET after single dose of G-CSF in group A was 6.46mm (SD ± 0.43) , minimum being 5.50 mm and maximum 7.50 mm. In group B, the mean ET was 7.28mm (SD ± 0.50) with a minimum of 5.50 mm and maximum figure of 8.00 mm. (Table 3)

This difference in the increase of ET between two groups comparing single and double dose of G-CSF was statistically significant with a p value of < 0.001, as deduced by Fisher's Exact Test. (Table 4)

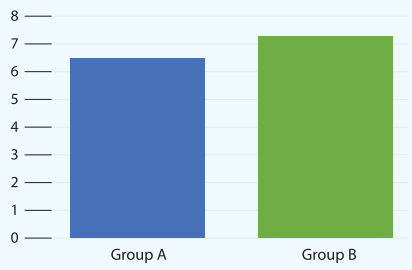

Table 3: Mean value of endometrial thickness at study end point

Endometrial thickness					
GROUP	Minimum	Maximum	Mean	Median	Std. Deviation
GROUP A	5.50	7.50	6.46	6.50	0.43
GROUP B	5.50	8.00	7.28	7.50	0.50
Total	5.50	8.00	6.83	6.75	0.62
p Value			<0.001		
Significance	Significant				

Table 4: Endometrial thickness and statistical analysis

Discussion

A successful pregnancy may be achieved when implantation of a competent blastocyst occurs into a receptive endometrium. Endometrial thickness is an important component of endometrial receptivity [8]. It is widely demonstrated that endometrial thickness of 7 mm or more is an essential prerequisite for a successful pregnancy [9,10]. For last several decades' different types of modalities have been used to achieve a desirable ET. Use of oral and topical estradial, low dose Aspirin, vaginal Sildenafil citrate, Pentoxyfilline and Tocoferol are some of the agents used for enhancement of ET [11,12,13].

Granulocyte colony stimulating factor(G-CSF) is a glycoprotein, which is synthesized by multiple hemopoietic cell types like fibroblast, macrophage and endothelial cells. It is also produced by several nonhematopoietic cell types, such as endothelial cells, placental cells, trophoblasts and granulosa-lutein cells [14,15]. Role of G-CSF has been established for enhancement of endometrial thickness, first reported by Gleicher and colleages in 2011[5,16]. Subsequently, larger studies and meta-analysis have supported the role of intrauterine instillation of this molecule in increasing ET in cases of thin endometrium[6,17]. On the contrary, some authors have concluded that there is no significant ET increase after G-CSF instillation[18]. There is contradictory evidence in the literature with regard to G-CSF induced endometrial expansion leading to higher implantation and biochemical pregnancy rates[7,19].

Role of subcutaneous injection of G-CSF have also been studied by Aleyasin et al [20], which showed significantly increase in implantation and pregnancy rates in infertile women with repeated IVF failure.

Most of the studies report the effect of G-CSF, using single dose.

This prospective cohort study was conducted to evaluate the effect of a second dose of intrauterine G-CSF instillation on thin endometrium and also to compare the effect of single dose and double dose of this molecule on thin endometrium.

150 women (aged between 23 to 40 years), planned for frozen-thawed embryo transfer (FET) were taken up for study. Patients were initially equally divided into group A, who received single dose of G-CSF and group B, where double dose has been administered. 3 patients in group A did not turn up for follow up and therefore 72 patients in this group were available for analysis. In group B, 2 patients were lost to follow up. Another 12 patients were excluded from study since they already had ET of 7 mm or more after first dose of G-CSF. Therefore 61 patients are left for analysis in group B. Finally, we had 133 patients in total.

In group A, 37 (51.4%) out of 72 women receiving single dose of G-CSF, had ET increase in the range of 6.5 to 6.9 mm after 72 hours. Minimum ET increase was 0.4mm and maximum was 2.4 mm in this group. 11 (15.28%) reached the desirable ET of 7 mm or more.

Gleicher et al (2011) [5], found that all 4 cases (100%)

in their study had ET of 7 mm or more and all of them conceived. Unlike our study, their pilot study was conducted on patients with fresh cycles.

Mishra et al[21], in FET cycle study on 35 patients documented ET increase of more than 7 mm in 19(54.28%) patients. In their series mean ET increased from 5.86mm ± 0.58 to 6.58mm ± 0.84 forty-eight hours after intrauterine G-CSF instillation. In our study ET increase was measured after 72 hours post instillation.

ET expansion after intrauterine G-CSF instillation was also reported by Miralaei et al[22], who demonstrated mean ET increase from 5.35± 1.06 to 6.52± 1.10. However, 45% patients in their series did not reach the desirable ET of 7mm.

In another study by Kunicki et al [6], ET increased from 6,74 ± 1.75 to 8.42 ± 1.73 mm 72 hours following infusion of G-CSF. But this study was undertaken in frozen ET cycle and the pretreatment ET was <7mm, contrary to our study where the pretreatment ET was 5.5mm.

In group B of our study, 39 (63.9 %) out of 61 patients receiving double dose of G-CSF had ET increase in the range of 7.5 to 7.9 mm. 10 patients (16.45%) achieved ET of 6.5 to 6.9 mm and 5 women (8.2%) ET raised to the range of 7 to 7.4 mm. Three patients (4.9%) achieved ET of more than 8mm. In Gleicher et al's study[16] of 21 patients , three (14.3%) reached the minimal thickness after the second infusion of G-CSF. But the figures after first and second dose of G-CSF was not compared in this study. Mishra et al [21] in their study on 35 patients undergoing FET, also administered second dose of G-CSF in 9 (25.7%) of patients, but comparative data was not available.

In present study, mean increase of ET after single dose of G-CSF in group A was 6.46mm (SD ± 0.43), minimum being 5.50 mm and maximum 7.50 mm. In group B, who received double dose, the mean ET was 7.28mm (SD ± 0.50) with a minimum of 5.50 mm and maximum figure of 8.00 mm. This difference in the increase of ET between two groups comparing single and double dose of G-CSF was statistically significant with a p value of < 0.001.

Eftekhar et al [18] studied the effect of two doses of intrauterine G-CSF in FET cycle on 34 patients and concluded that G-CSF does not improve ET, but has the potential to improve chemical and clinical pregnancies. But they infused the second dose 48 hours after the initial dose and we instilled the second dose 72 hours after the first dose. Similar conclusion was also drawn by Li J [7], who reported that G-CSF induced ET increase was not statistically significant, but documented significantly higher implantation and biochemical pregnancy rate.

Xie Y et al [17], on the other hand, reported in a meta-analysis a significant increase of ET after intrauterine G-CSF perfusion, associated with a decreased cycle cancellation rate.

Many adverse effects of G-CSF, in general have been reported in the literature namely bone pain, fatigue, headache, insomnia, anorexia, nausea or vomiting[23]. We however did not encounter any side effects in our series.

Conclusion

Intrauterine G-CSF instillation results in significant increase of ET in cases of persistently thin endometrium. Most of the patients reach close to the desired ET of 7mm after first dose. A second dose further enhances ET much beyond the minimum desired level in majority of patients. Double dose of G-CSF instillation leads to statistically significant increase of ET, compared to single dose.

Strength and Limitations of the Study

Though there are numerous studies on the effects of G-CSF on endometrial thickness, this series provides reasonably robust conclusion on the positive effect of G-CSF on thin endometrium. Furthermore, to the best of our knowledge, this is a unique study comparing

single versus double dose of G-CSF on a fairly large population.

Our study is not without limitations. We did not have a control group which received placebo. Thus, the changes of endometrial thickness could be observed only before and after infusion.

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