Original Article

Effect of intra uterine instillation of granulocyte colony stimulating factor for thin non responding endometrium during IVF-ET: Non randomized comparative study

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Abstract

Background: Implantation after embryo transfer is the final stage for the success of In vitro fertilization and embryo transfer (IVF-ET). Approximately 0.6-0.8 percent of patients do not reach minimum endometrial thickness for embryo transfer during IVF-ET. **Objective:** To study the effects of Granulocyte colony stimulating factor on endometrium and clinical pregnancy during IVF ET in patients with thin non responding endometrium. **Design, Setting, Participants:** A prospective comparative study comprising of 70 patients of IVF ET at a tertiary care hospital over a period of 18 months from Dec 2013 to May 2015. **Interventions:** Intra uterine instillation of granulocyte colony stimulating factor (GCSF) 300 µg/1ml to a group of patients with thin endometrium (< 7 mm) during IVF-ET versus no intervention to control group. **Main Outcome Measure:** Increase in Endometrial thickness (ET) on the day of embryo transfer and its effect on pregnancy rates. **Results:** Mean difference of ET between treatment group and control group during ovum pick up was 0.424 mm (P = 0.006) and 0.7286 (P = 0.0001) during embryo transfer which are statistically significant. Pregnancy rate in treatment group was 48.57% and 25.71% in control group. Cancellation of embryo transfer was 5.71% in treatment group and 40.00% in control group which is high (P=0.0017). **Conclusion:** Intra uterine instillation of GCSF during IVF-ET increases ET significantly in patients with thin non responding endometrium. It also improves pregnancy rate with significant decrease in cancellation of embryo transfer.

Keywords: IVF-ET, Granulocyte colony stimulating factor, Endometrial thickness.

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INTRODUCTION

Infertility is increasing with changes in life style and since the first reported birth of In vitro fertilization and embryo transfer (IVF ET) baby by Patrick Steptoe and Robert Edwards in the year 1978, lots of research have contributed to improve IVF outcome. Good implantation is the final stage for the success of IVF ET. There are many endometrial receptivity factors which affect the implantation. Endometrial thickness assessed by transvaginal ultrasound is the most commonly practiced method to assess the suitability of the endometrium for the embryo transfer. Approximately 0.6-0.8 percent of patients do not reach minimum endometrial thickness for embryo transfer.[1] There are multiple treatment modalities to improve endometrial thickness like Aspirin, Sildanafil, Estradiol and Pentoxiphylline,[2] but during our practice we have found many cases not responding to any of these modalities. Non responding endometrium is a challenge during IVF ET and to maximize pregnancy rates studies have suggested a minimal endometrial thickness of 7 mm,

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preferably above 10 mm but not more than 14 mm.[3, 4] Sharkey *et al* showed that immunological mechanisms in the endometrium are very important and crucial in the implantation process.[5] Some investigators demonstrated that the growth factors, hormones, and cytokines, which are produced by decidual cells, are involved in the implantation process.[6] Preliminary studies have demonstrated that Granulocyte colony stimulating factor (GCSF) stimulates neutrophilic granulocyte proliferation and differentiation, acts on macrophages of decidual cells and finally helps in the implantation.[7, 8] GCSF is reported to have effects on recruitment of dendritic cells, promoting Th-2 cytokine secretion, activating T regulatory cells, and also stimulation of various proangiogenic effects.[8,9] A potentially growth-expanding effects on endometrium may be suspected from its role in establishing early endometriosis lesions[10] and suppressing autoimmunity.[11] Options in patients with thin non responding endometrium during IV FET would be to cryopreserve embryos hoping for better endometrium in future, or to do embryo transfer and accept decreased implantation rate. Surrogacy may be considered as an option to patients with thin non responding endometrium. Presently surrogacy is under lots of legal dilemmas and is not an easy alternative. Recent pilot studies and some prospective studies have given hopes on the effect of GCSF for thin non responding endometrium during IVF ET. [2, 12] Hence this study was undertaken to further substantiate the effect of GCSF for thin non responding endometrium during IVF ET.

MATERIAL AND METHODS

Study design

A prospective comparative non randomized study comprising of 70 patients of IVF ET meeting inclusion and exclusion criteria were recruited in to the study over a period of 18 months from Dec 2013 to May 2015. The institutional ethical committee approved the study, and written informed consent was taken from participants.

Study Population

Recruited 70 patients undergoing IVF ET with the following inclusion and exclusion criteria's on the day of ovulation trigger.

Inclusion Criteria

- Lady undergoing IVF ET, aged between 21– 40 years.
- Documented thin endometrium (<7mm) during Intra uterine insemination cycle or previous IVF ET cycle.
- No associated uterine pathologies like Asherman's syndrome, fibroids, and intra uterine polyps.

Exclusion Criteria

- Age more than 40 years
- Cases with contraindications for G-CSF treatment (sickle cell disease, chronic neutropenia, renal insufficiency, pneumonia, and congenital fructose intolerance)

Study Interventions

Controlled ovarian stimulation for IVF ET was started with standard long GnRH agonist protocol. Follicular growth and ET (Endometrial thickness) were monitored throughout stimulation protocol with transvaginal sonography. After 06 doses of gonadotropin injections patients with follicular growth more than or equal to 15 mm and thin ET (</= 5mm) were considered for the study. Patients with thin ET (</= 5mm) were started on oral tablet estradiol valerate 2 mg three times a day as per institutional policy. Patients were considered for ovulation trigger with Human Chorionic Gonadotropin (HCG) when follicle size reached 18mm in diameter. 70 cases with ET less than 7 mm during ovulation trigger were recruited in to the study. Study group was divided in to treatment and control groups, each group having 35 patients (alternate patients were placed in to treatment and control group). Treatment group was administered intra uterine infusion of GCSF (300 µg/1ml) under ultrasound guidance with the help of embryo transfer catheter under aseptic conditions at the time of ovulation trigger and control group was not administered GCSF. Ovum pick-up was done 36 hours after ovulation trigger under guidance of transvaginal ultrasound. ET was reassessed at the time of ovum pick up and patients of treatment group with ET < 7 mm were re instilled an additional dose of GCSF. Study group was reassessed after 48 hours of ovum pick up for D2 embryo transfer. Patients with ET 7 mm and more underwent embryo transfer and embryos of patients with inadequate ET (< 7mm) were cryopreserved. After embryo transfer patients were given luteal phase support with daily micronized progesterone injection and estradiol valerate tablets for 16 days till the day of pregnancy test by serum β HCG test.

Procedure of GCSF instillation

- GCSF drug is aspirated into a 1-ml syringe and embryo transfer catheter is mounted on to 1ml syringe and the air is expelled.
- Embryo transfer catheter guide tip is placed till the level of internal os under ultrasound guidance with all aseptic precautions.
- Transfer catheter is passed in to uterine cavity as performed during an embryo transfer and the content of the syringe is slowly injected into the uterine cavity with utmost care to avoid endometrial injury.

Study Outcome

Primary outcome

To study the effects of Granulocyte colony stimulating factor on endometrium to increase endometrial thickness in patients with thin non responding endometrium during IVF ET.

Secondary outcome

To study the effects of Granulocyte colony stimulating factor on clinical pregnancy during IVF ET in patients with thin non responding endometrium.

Hypothesis for sample Size and Statistical Analysis

Hypothesis

Null Hypothesis: There is no significant improvement in treatment compared to Control Group. (H0: U1=U2).

Alternative Hypothesis: There is significant improvement or decline in treatment compared to Control Group. (H1: U1 Not Equal U2).

Estimation of Sample size

There were no comparative studies (Two Arm) was reported in literature determining the efficacy of treatment over control group, when this study was initiated. Only few publications were available as pilot studies through a single arm study and on small sample size. Hence this study was proposed as a pilot comparative two arm study in Indian population. As per our hospital documents on average 5-7 patients per month satisfying the exclusion and inclusion criteria are available. Taking this assumption and lost to follow up as 10-20 %, the study was proposed as a pilot study on a total no of 70 cases to be divided alternatively in treatment and control group.

Statistical Analysis

Details of all cases were recorded on a structured format and analyzed with the help of registered version of SPSS version 22. Group comparisons were made using independent *t*-test and paired t test. Statistical significance was assessed at $P< 0.05$.

RESULTS

Participant Flow Diagram

70 cases with ET less than 7 mm during ovulation trigger were recruited in to the study. Study group was divided in to treatment and control groups, each group having 35 patients. Treatment group was administered intra uterine infusion of GCSF (300 µg/1ml) during ovulation trigger and control group was not administered GCSF.

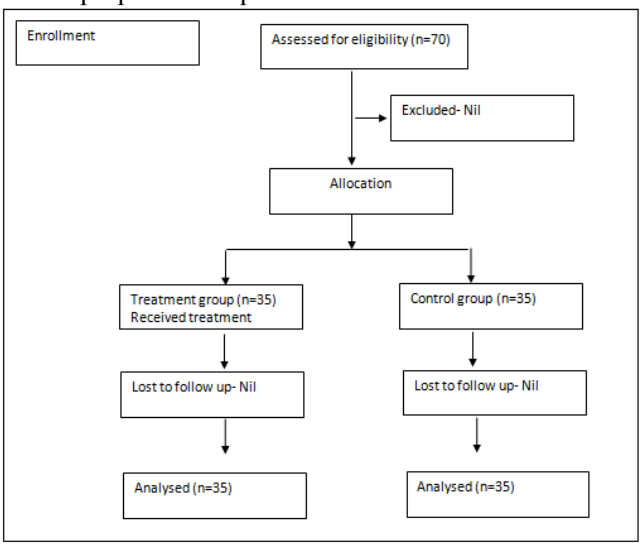

Table 1: Descriptive data

Descriptive	Treatment Group	Control Group	p-value
Age (Mean)	29.143	30.03	.342
Duration of Infertility (Mean)	8.257	9.143	.271
ETIOLOGY OF INFERTILITY			
Unexplained	18 (51.4%)	19 (54.28%)	0.9985
Genital Tuberculosis	04 (11.4%)	05 (14.285 %)	0.9972
Male factor	05 (14.285 %)	03 (8.57%)	0.7071
B/L tubal block	02 (5.71%)	02 (5.71%)	0.6065
Decreased Ovarian Reserve	01 (2.85%)	05 (14.285 %)	0.1998
PCOD	04 (11.4%)	01 (2.85%)	0.3546
Fibroid uterus	01 (2.85%)	-	

In our study mean age (Table No 1) of treatment group was 29 years and control group age was 30 years which are comparable. This is further ascertained by p-value of 0.342 which suggests no significant difference in age between the two groups. Mean duration of infertility among treatment group was 8.25 years and control group was 9.143 years and the difference is again not significant (P=0.271) between two groups. Unexplained infertility (Table No 1) was the most common etiology of the infertility amongst the study group, it was 51.4% in the treatment group and 54.28% in the control group and both were comparable for the etiological aspects. Second most common etiology was genital tuberculosis which may affect the endometrium, patients suspected to have genital tuberculosis were given empirical anti tuberculosis treatment for 06 months. Cases of end organ damage and Asherman's syndrome were not included in the study and again this etiology was comparable in both the groups. Other etiological aspect of ovary like poly cystic ovarian syndrome and decreased ovarian reserve included in the study but the difference between the two groups was not statistically different.

Table 2: Mean Values of Endometrium Thickness at Different Stages of IVF-ET

Descriptive	Treatment Group			Control Group			Mean Difference	P value
	Endometrial thickness (ET) (mean in mm)	95% Confidence Interval of the Difference		Endometrial thickness (ET) (mean in mm	95% Confidence Interval of the Difference			
		Lower	Upper		Lower	Upper		
ET during starting of Estradiol valerate tablet	4.669	4.567963769	4.769179088	4.829	4.740656517	4.956486341	0.180	0.020
ET during Ovulation Trigger	5.637	5.441458405	5.832827309	5.400	5.29709902	5.50290098	0.2371	0.040
ET during ovum pick up	6.424	6.189388851	6.659182578	6.000	5.822314488	6.177685512	0.424	0.006
ET During Embryo transfer Treatment group	7.206	7.007105762	7.40432281	6.477	6.232090392	6.722195322	0.7286	<.0001

On analyzing data from table 2 and 3 on the day of ovulation trigger our study group showed increase in the endometrial thickness. Mean increase in ET in Treatment group was 0.9686 mm and 0.5514 mm in control group with a statistical significance of <0.0001 in both groups. On the day of ovum pick up 48 hours after GCSF instillation mean increase in ET in treatment group was 1.7557 mm with a statistical significance of <0.0001 and in control group mean increase was 1.1514 mm with a statistical significance of <0.0001. On comparing ET within each study group the increase in ET is significant in both groups. But on comparing mean difference between treatment and control group, mean difference was 0.424 which states increase in ET is better in treatment group with a statistical significance of 0.006. During embryo transfer the mean difference in the endometrial thickness between treatment group and control group was 0.7286 which was statistically significant with a p value of <0.0001.

Table 3: Paired Differences of Endometrium Thickness at Different Stages

Descriptive	Paired Differences									
	Treatment Group					Control Group				
	Mean	Std. Deviation	95% Confidence Interval of the Difference		P-Value	Mean	Std. Deviation	95% Confidence Interval of the Difference		P-Value
			Upper	Lower				Upper	Lower	
ET difference from starting of Estradiol valerate tablet to ovulation trigger	.9686	.4206	1.1131	.8241	<.0001	.5514	.2944	.6526	.4503	<.0001
ET difference from starting of Estradiol valerate tablet to ovum pick up	1.75571	.53862	1.94074	1.57069	<.0001	1.1514	.5425	1.3378	.9651	<.0001
ET difference from starting of Estradiol valerate tablet to embryo transfer	2.5371	.5440	2.7240	2.3503	<.0001	1.6286	.7540	1.8876	1.3696	<.0001
ET difference from ovulation trigger to ovum pick up	.78714	.34457	.90551	.66878	<.0001	.6000	.3678	.7264	.4736	<.0001
ET difference from ovulation trigger to embryo transfer	1.5686	.5010	1.7407	1.3965	<.0001	1.0771	.6112	1.2871	.8672	<.0001
ET difference from ovum pick up to embryo transfer	.78143	.56648	.97602	.58684	<.0001	.4771	.3623	.6016	.3527	<.0001

Pregnancy rate in the treatment group was 48.57% and 25.71% in the control group (Table 4). Clinically pregnancy rate among treatment group was high when compared to control group but it was statistically not significant (p=0.0833). Negative pregnancy rate in the treatment group was 45.71% and 34.29% in the control group. Though the negative pregnancy in treatment group was high when compared to control group but it was statistically not significant (P=0.4647). Cancellation of embryo transfer in the treatment group was 5.71% and 40.00% in the control group. 60% of the patients in the treatment group required second dose of GCSF.

Table 4: GCSF effect on pregnancy outcome and Cancellation of embryo transfer

Descriptive	HCG Result of study group (N=70)		HCG Result Treatment Group		HCG Result Control Group		P-Values
	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Cancellation of Embryo transfer	16	22.86%	2	5.71%	14	40.00%	0.0017
Negative Pregnancy	28	40.00%	16	45.71%	12	34.29%	0.4647
Positive Pregnancy	26	37.14%	17	48.57%	9	25.71%	0.0833
Total	**70**	**100.00%**	**35**	**100.00%**	**35**	**100.00%**	

DISCUSSION

A good and receptive endometrium is the most important requisite for the successful implantation. In approximately 1% of IVF cycles the endometrium is found to be thin and inadequate for implantation. Non responding endometrium is a challenge during IVF-ET. Studies have suggested a minimal endometrial thickness of 7 mm, preferably above 10 mm but less than 14 mm[3, 4] for optimal pregnancy rates. During our clinical practice we have experienced that many known treatment modalities fail to improve the endometrial thickness and non responding thin endometrium becomes a real challenge. In our study mean age of treatment group was 29 years and control group was 30 years which were almost comparable. Mean age of the patients in a similar study by David H. Barad *et al* was 39.59 years, which concluded that perfusion of the endometrial cavity with GCSF in women with normal endometrial proliferation does not offer any clinical benefit.[13] This negative result could be because patients age group in the study of David H. Barad *et al* was high and they studied the effect on patients with normal endometrium.

Endometrial response

Intervention for the thin endometrium was started with tablet estradiol valerate when antral follicular size was more than or equal to 15 mm. On day of ovulation trigger increase in ET in Treatment group was 0.9686 mm and 0.5514 mm in control group with a statistical significance of <0.0001 in both group. Our study demonstrates the positive effect of oral Estradiol valerate on improving the endometrial thickness which is in agreement with the study by N. Gleicher *et al*.[2] Intra uterine GCSF instillation was done on the day of ovulation trigger and ET was reassessed 48 hours later on day of ovum pick up. On day of day of ovum pick up mean increase in ET in treatment group was 1.7557 mm and 1.1514 mm in control group with a statistical significance of <0.0001 in both groups. Mean difference between treatment and control group on the day of ovum pick up was 0.424 which implies that increase in ET is better in treatment group with a statistical significance of 0.006. This finding is in agreement with the finding of a pilot study by N. Gleicher *et al* which demonstrate a significant improvement in endometrial thickness after G-CSF treatment.[2] In another study by Michal B Kunicki *et al*[14] the mean difference of ET increase was 1.68 ± 1.05 mm, it is almost similar to our study which is 1.7557 mm. Increase in ET was statistically significant in both the studies with p value of 0.001 and 0.0001 respectively. Another study by Ensieh Tehraninejad *et al* has demonstrated the mean difference between past cancelled cycle and studied GCSF cycle was 1.36 ± 1.1 mm, p value = 0.001[15]. However a randomized controlled study by David H Barad *et al*[13] and a non randomized two arm comparative study by Maryam Eftekar *et al*[16] contradicts our study and suggests that GCSF has no significant beneficial effect on endometrium. Mean difference in the endometrial thickness during embryo transfer between treatment group and control group was 0.7286 (P=<0.0001). This significant increase in ET could be reason for better implantation and pregnancy rate in patient treated with GCSF instillation. In our study 60% of the patient's required second dose of GCSF instillation where as in the study by N. Gleicher *et al* repeat dose was required in 14.3% of cases.[2] This suggest that assessment of endometrium is very important during Ovum pick up and remedial measures to improve endometrium can be tried.

Implantation and clinical pregnancy

In our study overall implantation and pregnancy rate was 37.14% and it was 48.57% in the treatment group which is equal to normal implantation rate of our IVF centre and implantation rate of control group was 25.71%. As per the initial case series of N. Gleicher *et al* implantation rate of 4 cases was 100% (12) and his subsequent pilot study showed clinical pregnancy rate of 19.1%[2]. Pregnancy rate was 20% in the study of Ensieh Tehraninejad *et al*.[15] and it was 18.9% in a study by Michal B Kunicki *et al*.[14] Our

study suggests that instillation of GCSF to thin non responding endometrium during IVF ET improves implantation and pregnancy by nearly 23% but it is statistically not significant (P=0.0833) but it seems to be appreciable clinically. A similar improvement in pregnancy rate was also seen in the study by Maryam Eftekar *et al* [16]

Cancellation of embryo transfer and Negative pregnancy

Embryo transfer was cancelled when ET was < 7 mm at the time of embryo transfer and embryos were cryopreserved. Cancellation of embryo transfer in treatment group was 5.71% and 40.00% in control group. Cancellation of embryo transfer was more in the control group and it was statistically significant (p=0.0017). It conveys that GCSF definitely helps thin non responding endometrium patients and prevents cancellation of embryo transfer. As per the study by Ensieh Tehraninejad *et al* cancellation of embryo transfer was 13.3%[15]. Where as it is 15.20% as per the study by Maryam Eftekar *et al* [16]. Negative pregnancy rate in treatment group was 45.71% and 34.29% in the control group. Though the negative pregnancy seems to be more in the treatment group the difference is statistically not significant (p=0.4647). Instillation of GCSF for thin non responding endometrium is recommendable as the benefits outweigh the risks.

CONCLUSION

Thin non responding endometrium is a challenging clinical situation during IVF-ET. Our study concludes that intra uterine instillation of GCSF during periovulatory period increases endometrial thickness significantly. GCSF instillation improves implantation rate and clinical pregnancy rate. It also significantly decreases cancellation of embryo transfer in cases of thin non responding endometrium during IVF-ET. Limitations of the Study: Not a randomized trial

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