ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 04 | April - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

THE EFFECT OF INTRAUTERINE INSTILLATION OF G-CSF IN INFERTILE WOMEN WITH THIN ENDOMETRIUM IN IUI CYCLE

Obstetrics & Gynaecology

Kumari Shashi Senior Resident, Department of Reproductive Medicine, Indira Gandhi Institute Of Medical Sciences, Patna Bihar, India.

Kalpana Singh* Additional Professor and Head, Department of Reproductive Medicine, Indira Gandhi Institute Of Medical Sciences, Patna Bihar, India.*Corresponding Author

Bhawana Tiwary Assistant Professor, Department of Reproductive Medicine, Indira Gandhi Institute Of Medical Sciences, Patna Bihar, India

Shubhanti Kumari Assistant Professor, Department of Reproductive Medicine, Indira Gandhi Institute Of Medical Sciences, Patna Bihar, India

Huma Nishat Assistant Professor, Department of Reproductive Medicine, Indira Gandhi Institute Of Medical Sciences, Patna Bihar, India

ABSTRACT

Context: The normal thickness of endometrium is 7 to 14 mm in secretory phase and it is a prominent factor for successful pregnancy. In this study, impact of G-CSF infusion on thin endometrium and role in pregnancy outcome was studied. **Aims:** To study the effect of intrauterine instillation of G-CSF in infertile women with thin endometrium in IUI cycle. **Design:** It was a hospital based prospective study, done for 1 year (March 2018 - February 2019) in the department of Reproductive Medicine, IGIMS ,Patna. **Methods:** Fifty patients in IUI cycle , having endometrium ≤7 mm on the day of trigger, received intra uterine instillation of G CSF (300 mcg/ml) using IUI catheter. ET was reassessed after 36 hours by ultrasound on day of IUI. Urine pregnancy test was done after 18 days. **Statistical analysis:** It was done by descriptive and inferential statistics using student's paired t test and software used are SPSS 22.0 version and Graph-Pad Prism 6.0 version **Results:** ET improved in all the patients. Mean ET before infusion was 5.6840 ±0.44371 mm and 36 hours after infusion was 8.1000 ±0.40507 mm. Difference in ET was 2.41± 0.29441(Significant). UPT was positive in 8 out of 50 patients. **Conclusions:** G-CSF intrauterine infusion has the potential to improve endometrial thickness and pregnancy outcomes in IUI Cycle

KEYWORDS

G-CSF – Granulocyte colony stimulating hormone, ET – Endometrial thickness, IUI- Intrauterine insemination., UPT –Urine pregnancy test

INTRODUCTION

Healthy seed is not enough to get a healthy sapling unless it is grown on fertile soil. Similarly healthy embryo needs receptive endometrium for successful implantation[1]. The endometrium is said to be receptive when it supports the transformation of endometrial cells into decidual cells, promotes invasion of blastocysts, and causes growth of placenta for continuation of successful pregnancy[2].For evaluating endometrial receptivity various methods like the histological dating of an endometrial biopsy, the genomic study of a timed endometrial biopsy ,endometrial cytokines from fluid obtained from uterine flushing or ultrasound examination of the endometrium can be used.

Review of literature

Many studies had shown that endometrial thickness and endometrial pattern as prognostic parameters for determining endometrial receptivity. But successful evaluation of endometrial receptivity conducive to embryo implantation is still a challenge to assisted reproductive technology. Ultrasonography is the most commonly used techniques as it is non-invasive and atraumatic. The endometrium is usually best seen on transvaginal sonography where the endometrial thickness and pattern can be assessed. The normal thickness of endometrium in secretory phase is 7 to 14 mm and for successful implantation it is very important factor[3,4].Studies have shown that there is less chance of pregnancy if endometrium thickness is less than 6mm[5,6].Improving endometrial growth in patients having thin endometrium is very difficult process. Therefore chances of successful pregnancy in these patients is also reduced. There have been various methods to improve endometrial thickness, some traditional methods like low dose aspirin, pentoxfylline, tocopherol, ,arginine, estrogenand vaginal sildenafil are administered. Many a times administration of these agents is ineffective or some methods need longer duration of treatment for effective increase in thickness so recently newer methods like Granulocyte colony- stimulating factor intrauterine instillation , Endometrial scratching before embryo transfer , Stem cell therapy and Platelet rich plasma instillation have been tried to achieve appropriate thickness of endometrium.

G-CSF infusion on unresponsive thin endometrium and pregnancy outcome. Immunological mechanisms of the endometrium plays a very important role in implantation[7] .Granulocyte colony stimulating factor (G-CSF) is a hematopoietic cytokine that is found in Feto-maternal interface during embryo implantation and early pregnancy suggesting that it may have a role in decidua and placental development [8]. It is believed that G-CSF has important function in follicular maturation, ovulation, implantation and pregnancy after conception.It promotes granulocyte proliferation,differentiation and maturation[9]. G-CSF administration in patients with recurrent spontaneous pregnancy losses and repetitive implantation failures is suggested to improve pregnancy outcomes[10-12] .G-CSF causes recruitment of neutrophils and release of vascular endothelial growth factor that leads to vascular remodeling ,endometrial growth, uterine gland transformation growth of new blood vessels this ultimately improves uterine blood supply, endometrial decidualization and endometrial thickening. It is produced by recombinant DNA technology from E- coli into which the human G-CSF gene has been inserted.

Although many trials have been in favour for using G – CSF in thin endometrium, it is still a new remedy. Effective dose, the day on which it is to be instilled and the efficacy of G-CSF in improving endometrium, and possibly pregnancy rates, needs to be confirmed further by randomized controlled trials. G – CSF is a type of colony stimulating factor produced by different tissues in body like endothelium, macrophages and a number of immune cells. It plays important role in follicular development, ovulation and implantation of embryo[13]. It is proposed that G-CSF may stimulate endometrial stem cells or mobilize bone marrow stem cells promoting growth of endometrium. Gleicher et al. demonstrated that G-CSF is a novel remedy in infertile patients with unresponsive and thin endometrium.[14] In that study intrauterine G-CSF was used in four patients undergoing IVF with thin endometrium. In all four patients endometrial thickness was found to improve and they underwent embryo transfer and finally conceived. So they concluded that G-CSF treatment improve IVF pregnancy chances. In subsequent pilot cohort study by Gleicher N et al., 21 women with thin endometrium were evaluated. After instilling G-CSF, endometrial thickness increased from 6.4 ±1.4 to 9.3 ±2.1 mm by the transfer day which was statistically significant. Pregnancy rate in this study was only19perecent[5].

Barad et.al concluded that G-CSF has no effect on endometrial thickness, implantation rates and clinical pregnancy rates.[9] Prospective study by Mishra V et al. only 52% patient showed increase

in endometrial thickness and three patients (15.78%) had biochemical pregnancies but none of them had clinical pregnancy[15].Kamath et.al. analysed different studies and concluded that Granulocyte colony stimulating factor is involved in different reproductive functions, like ovulation, decidualization, endometrial regeneration and endometrial receptivity. Also G-CSF helps to increase the endometrial thickness in patients with thin endometrium. But they also pointed the need for controlled randomized trials to establish the role of G-CSF in ART practice[16].Randomised controlled trail done by M. Kunicki et.al. and Fatemeh Sarvi et. al. concluded that the use of G CSF in patients with thin endometrium increases endometrial thickness[17,18].Randomised controlled trail in 2016 done by Maryam Eftekhar, et.al. concluded that in normal IVF patients with normal endometrial thickness, the intrauterine infusion of G-CSF did not improve pregnancy outcomes[19].Whereas Xie Y et.al. did a meta-analysis in 2017 which included 11 studies (683 patients) and concluded that G-CSF instillation could significantly improve endometrial thickness, clinical pregnancy rate and embryo implantation rate ,while it decreased cycle cancellation rate (RR=0.38, 95% CI: 0.25-0.58) as compared to control group[20].Thus there have been many trials in favor for using G – CSF in thin endometrium, but still it is a new remedy in our population . The effective dose of G-CSF, day on which it is to be instilled, its efficacy in improving endometrial thickness, and possibly pregnancy rates after G-GCSF instillation needs to be confirmed in further randomized controlled trials. In the present study, we studied the impact of intrauterine G-CSF instillation on thin endometrium and its role in pregnancy outcome.

METHODS

The aim of our study was to study the effect of intrauterine instillation of G-CSF in infertile women with thin endometrium.

The objectives of our study : Primary objective: To study the effect of granulocyte colony stimulating factor on endometrium in infertile women planned for IUI with thin endometrium (<7mm) and Secondary objective : To study the pregnany outcome after giving G CSF in infertile women planned for IUI with thin endometrium (<7mm)

Study design and data collection: This was a hospital based prospective study; carried out in the Department of Reproductive Medicine, IGIMS, Patna ,Bihar for one year duration (March 2018 - February 2019)on 50 infertile patients attending outpatient department for treatment.

- **Inclusion criteria:** All infertile women of age group 18-35yr ,with previously failed IUI due to thin endometrium and inadequate endometrial thickness that was less than 7 mm when size of dominant follicle is more than 18 mm
- **Exclusion criteria:** Patients not giving consent for study , patient with intrauterine adhesions or polyps or sub mucous fibroids ,patients with contraindication for use of granulocyte colony stimulating factor (like hypersensitivity, any past medical condition eg. sickle cell disease, renal insufficiency) and patient suffering from chronic illness , HIV/hepatitis patients.

Study methodology

Purpose of the study was explained to the patients and their written consent was taken prior to the study**.** Women suffering from infertility were evaluated on the basis of predesigned and pretested proforma with respect to history, clinical examination and investigations. Women suffering from infertility who were planned for treatment with IUI cycles, were selected. Ovulation induction was given on Day 2 for five days of menstrual cycle .Serial TVS evaluation for folliculometry and endometrial thickness was done from Day 9. Patients with endometrial thickness less than 7 mm on the day of trigger (when follicular size ≥ 18 mm) were selected for intrauterine G-CSF instillation. Intrauterine instillation of G-CSF (300 mcg/ml)was done slowly by using IUI catheter on the day of trigger. 36 hours after instillation, the endometrial thickness was reassessed by TVS with the same machine. Then IUI was done 36 hour after trigger. Urine pregnancy test was done 18 -20 days after IUI . Result was noted as positive or negative.

The primary objective was to see the increase in endometrial thickness and secondary objective was to see result of urine pregnancy test. We divided the patients in two groups depending on the whether urine pregnancy test was positive or negative. Statistical analysis was done by using descriptive and inferential statistics using student's paired t test and software used in the analysis are SPSS 22.0 version and Graph-Pad Prism 6.0 version and $p<0.05$ was considered as level of significance.

RESULTS

In our study out of 50 patients, 24 patients (48 %) were between 25 to 29 years of age. Among these patients 56% patients (28) had primary infertility and 44% patients (22) had secondary infertility. Average duration of infertility of these patients was 3.5360 ± 1.36243 years. (Table 1,2).

TABLE 1: BASIC PARAMETERS OF THE PATIENTS IN THIS STUDY

PARAMETERS	ALL PATIENTS
Mean age	27.4800 ± 4.05191 years
Average duration of infertility	3.5360 ± 1.36243 years
Primary infertility	56%
Secondary infertility	44%

TABLE 2: DISTRIBUTION OF PATIENTS ACCORDING TO AGE

	Total Number of Patients	Minimum age in years	Maximum Age in years	Mean	Standard deviation
Age	50	20.00	36.00	27.4800	4.05191

The average endometrial thickness pre infusion was 5.6840±.44371 mm, which increased to 8.1000± 40507 mm post infusion The difference was 2.41± 0.29441 mm. statistically significant difference was found in mean endometrial thickness before and after infusion (p-value 0.0001S). (TABLE 3)

TABLE 3: ENDOMETRIAL THICKNESS VARIATIONS WITH TREATMENT

Endometrial thickness (ET)	Min Thickness (mm)	Max Thickness (mm)	Mean ET (mm)	Std. Deviation	Increase in ET (mm)	Significance (p value)
On the day of trigger	4.80	6.60	5.6840	.44371	2.41± 0.29441	.0001
On the day of IUI	7.20	9.00	8.1000	.40507		

The minimum value of endometrial thickness on day of trigger was 4.8 mm and after treatment with G-CSF ,on the day of IUI it was found to increase to 7.2mm.Also the maximum endometrial thickness recorded in study patients on the day of trigger was 6.6 mm and it also increased after G-CSF treatment .(FIGURE1)

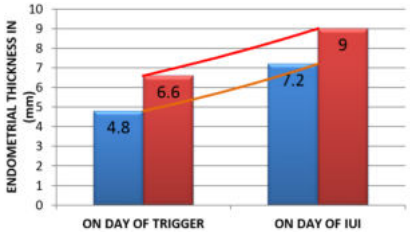

Figure 1: Showing Difference In Endometrial Thickness On The Trigger (before GCSF Instillation) And On The Day Of IUI(After GCSF Instillation)

In our study only 8 patients out of 50 i.e. 16 % showed positive urine pregnancy test.(TABLE 4)

Table 4: Distribution Of Patients According To Urine Pregnancy Test Result

INFERTILITY	PREGNANCY		TOTAL PATIENTS
	NEGATIVE	POSITIVE	
PRIMARY	23	5	28
	(82.14%)	(17.86%)	
SECONDARY	19	3	22
	(86.36%)	(13.63%)	
TOTAL		8 (16%)	50

DISCUSSION

The G-CSF seems to be promising treatment option in infertile women with thin endometrium showing significant improvement of endometrial thickness after intrauterine instillation of G-CSF.

The dose of GCSF used for intrauterine infusion in our study was 300 mcg/1 ml similar to that in Gleicher et al.[5], Mishra et al.[15] Kunicki et al.[17], Bin Xu et al.[21],Kim YY et al.[22],Tehraninejad et al.[23],Shah et al.[24]andAleyasin et al.[25]study . In a study conducted by Gleicher et al.[5], 3 out of 21 (14.35 %) patients received second infusion of G-CSF. Li et al[26]. used only 100 µg/0.6 ml G-CSF. Estradiol valerate was used for priming the endometrium before giving G-CSF by Li et al, Shah et al. and Bin Xu et al.

In our study we found that the average endometrial thickness pre infusion was 5.6840±.44371 mm, which increased to 8.1000 ± 40507 mm post infusion. The difference was 2.41± 0.29441 mm which was statistically significant difference (p-value 0.0001S).Gleicher et al.[14] found that there was increase in endometrial thickness > 7mm in all cases. In another study Gleicher et al[5] demonstrated significant increase in ET 2.9 ± 2 mm . Study done by Kim YY et al[22] found Increase in ET by 2.5 ± 1.2 mm, Kunicki et al[17] concluded that endometrial thickness increased by 1.68 ± 1.05 mm .In Shah et al. study ,average increase in endometrial thickness was 2.5 mm after 4 days of G-CSF infusion. Bin Xu et al[21] study stated that increase in ET was 2.4 ± 1.4 mm .Tehraninejad et al[23] concluded that increase in ET by 3.5 ± 0.88 mm which was a significant value. Li et al in a recent meta-analysis included 11 studies and 683 patients concluded that G-CSF perfusion could significantly improve endometrial thickness. But some studies like in Eftekharet al.[19]study, no improvement in endometrial thickness was noted after G-CSF intrauterine instillation. Also in Aleyasin et al.[25] study and Barad et al. study , difference in endometrial thickness in study and control group was similar, so they concluded that the use of G-CSF was ineffective in increasing the endometrium thickness among infertile women undergoing IVF. Mishra et al.[15]study concluded that in only 52% cases endometrial thickness increased to >7 mm.

In our study only 8 patients out of 50 i.e. 16 % were pregnancy positive after using G-CSF on the day of trigger .This was similar to Mishra et al.[15] study (pregnancy rate of 15.1%) and Kunicki et al.[17] study (18.9% pregnancy rate) . Pregnancy rate was higher after treatment in Gleicher et al.[5] study(pregnancy rates 19.1%), Bin Xu[21] et al. study (pregnancy rate 48.1%), Kim YY[22] et al. study (29.4% in patients with poor endometrium), Tehraninejad et al.[23] study (20% pregnancy rate) ,Shah et al[24]. study (37% in thin endometrium), and Aleyasin et al[25]. study (44.6% pregnancy rate). According to Xie Y et al.[20] meta-analysis that included 11 studies concluded that G-CSF significantly improved endometrial thickness, clinical pregnancy rate and decreased cycle cancellation rate as compared to control group in IVF cycles. But Barad et al.[9] and Li et al.[26] study, no difference was noted in implantation rates and pregnancy rates in study and control group.

Unlike all previous studies we used G-CSF in thin endometrium of patients on the day of trigger in IUI cycles instead of IVF cycles.

CONCLUSION

Even today improving endometrial growth in women with a thin endometrium is a challenge for assisted reproductive techniques. Despite many treatment options we still need acceptable, potent and effective remedy. Infusion of G-CSF in endometrial cavity is a safe and probably effective method to increasing endometrial thickness. We found that there was significant increase in endometrial thickness after G-CSF intrauterine instillation in women with a thin endometrium on the day of trigger and it may enhance the pregnancy and implantation rate in women with a thin endometrium even in IUI cycles. In this aspect, G-CSF instillation could be used as a strategy to induce adequate endometrial growth and good pregnancy results.

The effectiveness of G-CSF instillation should further be confirmed via well-designed and controlled studies.

DECLARATIONS

Funding: *NA*

Conflict of interest: *None*

Ethical approval: Departmental Research Committee, Institute Ethical Committee, IGIMS, Patna, Bihar

REFERENCES

1. Salamonsen LA, Nie G, Hannan NJ, Dimitriadis E (2009) Society for Reproductive Biology Founders' Lecture 2009. Preparing fertile soil: the importance of endometrial receptivity. Reprod Fertil Dev 21: 923–934.
2. Zenclussen AC, Hämmerling GJ. Cellular regulation of the uterine microenvironment that enables embryo implantation. Front inimmunol.2015; 6:321.
3. Senturk LM, Erel CT. Thin endometrium in assisted reproductive technology. Curr Opin Obstet Gynecol, 2008; 20(3): 221-228.
4. Loke YW, King A, Burrows TD. Decidua in human implantation. Hum Reprod. 1995 Dec;10 Suppl 2:14-21.
5. Gleicher N , Kim A, Michaeli T, Lee HJ, Shohat-Tal A, Lazzaroni E, Barad DH. A pilot cohort study of granulocyte colony stimulating factor in treatment of unresponsive thin endometrium resistant to standard therapies. Hum Reprod. 2013;28(1):172-7.
6. Kunicki M, Łukaszuk K, Woclawek-Potocka I, Liss J, Kulwikowska P, Szczyptańska J.Evaluation of Granulocyte Colony-Stimulating Factor Effects on Treatment-Resistant Thin Endometrium in Women Undergoing In Vitro Fertilization .Biomed Res Int. 2014;2014:913235
7. Sharkey A. Cytokines and implantation. Rev Reprod .1998; 3(1):52-61.
8. G-CSF for infertile women with thin endometrium in frozen ET program:A non-randomized clinical trial. Iran J Reprod Med. 2014; 12(10):661-666.
9. Barad DH, Yu Y, Kushnir VA, Shohat-Tal A, Lazzaroni E, et al. A randomized clinical trial of endometrial perfusion with granulocyte colonystimulatingfactor in in vitro fertilization cycles: impact on endometrialthickness and clinical pregnancy rates. Fertil Steril. 2014 Mar;101 (3):710-5.
10. Santjohanser C, Knieper C, Franz C, Hirv K, Meri O, et al. Granulocyte colony stimulating factor as treatment option in patients with recurrent miscarriage. Arch Immunol Ther Exp (Warsz). 2013 Apr; 61(2):159-64.
11. Scarpellini F, Sbracia M. Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: a randomised controlled trial. Hum Reprod. 2009; 24(11):2703-8.
12. Würfel W. Treatment with granulocyte colony-stimulating factor in patients with repetitive implantation failures and/or recurrent spontaneous abortions. J Reprod Immunol. 2015;108: 123-35.
13. K. Yanagi S, Makinoda R, Fujii S, Miyazaki S, Fujita H, Tomizawa K, Yoshida T, Iura T, Takegami T, Nojima T .Cyclic changes of granulocyte colony-stimulating factor (G-CSF) mRNA in the human follicle during the normal menstrual cycle and immunolocalization of G-CSF protein. Hum Reprod. 2002; 17(12):3046-52.
14. Gleicher N, Vidali A, Barad DH. Successful treatment of unresponsive thin endometrium. Fertil Steril .2011; 95(6):2123.
15. Mishra V, Choudhary S, Sharma U, Aggarwal R, Agarwal R, Gandhi K, Goraniya N. Effects of granulocyte colony-stimulating factor (GCSF) on persistent thin endometrium in frozen embryo transfer (FET) cycles. J Obstet Gynaecol India. 2016 oct; 66(Suppl 1)407-411.
16. Kamath MS, Chittawar PB, Kirubakaran R, Mascarenhas M. Use of granulocyte-colony stimulating factor in assisted reproductive technology: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2017 Jul; 214:16-24.
17. Michał Kunicki, Krzysztof Łukaszuk, Joanna Liss, Patrycja Skowrońska. Granulocyte colony stimulating factor treatment of resistant thin endometrium in women with frozen-thawed blastocyst transfer. Journal of Obstetrics &Gynecology .Syst Biol Reprod Med. 2017 Feb; 63(1):49-57.
18. Fatemeh Sarvi, Marjan Arabahmadi, Ashraf Alleyassin, Marzieh Aghahosseini, and Marzieh Ghasemi .Effect of Increased Endometrial Thickness and Implantation Rate by Granulocyte Colony-Stimulating Factor on Unresponsive Thin Endometrium in Fresh In Vitro Fertilization Cycles: A Randomized Clinical Trial. Obstetrics and Gynecology International. 2017; Article ID 3596079.
19. Maryam Eftekhar, Robabe Hosseinisadat, Ramesh Baradaran, Elham Naghshineh. Effect of granulocyte colony stimulating factor (G-CSF) on IVF outcomes in infertile women: An RCT.Int J Reprod Biomed (Yazd). 2016 May; 14(5): 341–346.
20. Xie Y, Zhang T, Tian Z, Zhang J, Wang W, Zhang H, Zeng Y, Ou J, Yang Y.Efficacy of intrauterine perfusion of granulocyte colony-stimulating factor (G-CSF) for Infertile women with thin endometrium: A systematic review and meta-analysis .Am J Reprod Immunol. 2017; 78(2).
21. Xu B, Zhang Q, Hao J, Xu D, Li Y. Two protocols to treat thin endometrium with granulocyte colony-stimulating factor during frozen embryo transfer cycles. Reprod Biomed Online.2015; 30(4):349-358.
22. Kim YY, Jung YH, Jo JD, Kim MH, Yoo YJ, Kim S. The effect of transvaginal endometrial perfusion with granulocyte colony-stimulating factor (G-CSF). Fertil Steril.2012; 98(3): S 183
23. Tehraninejad E, Enieh MD, Davari F, Asadi E, Kamali K, Koorosh MD, Aziminikoo E, Elham MD, Rezavof E. G-CSF intrauterine for thin endometrium, and pregnancy outcome. J Family and Reprod Health. 2015; 9(3):107-112.
24. Shah J, Gangadharan A, Shah V. Effect of intrauterine instillation of granulocyte colony-stimulating factor on endometrial thickness and clinical pregnancy rate in women undergoing in vitro fertilization cycles: an observational Cohort study. Int J Infertil Fetal Med .2014; 5(3):100-106.
25. Aleyasin, A., Abediasl, Z., Nazari, A., Sheikh, M. Granulocyte colony stimulating factor in repeated IVF failure, a randomized trial. Reproduction .2016;151(6):637-642.
26. Li Y, Pan P, Chen X, Li L, Li Y, Yang D. Granulocyte colony stimulating factor administration for infertile women with thin endometrium in frozen embryo transfer program. Reprod Sci. 2014 ; 21(3):381-385.