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Original Research Article

Intrauterine instillation of granulocyte colony stimulating factor for infertile women with thin endometrium in intrauterine insemination cycle: a non-randomized clinical trial

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ABSTRACT

Background: Endometrial thickness is one of the major factors for a receptive endometrium and successful implantation. Thin endometrium, generally measuring <7 mm are thought to be less able to support implantation and pregnancy. Several adjuvants have been investigated for their efficacy on thin endometrium in assisted reproductive technology. Granulocyte colony stimulating factor (G-CSF) is a glycoprotein that promotes endometrial stromal cell decidualization via cyclic adenosine monophosphate mediator and induces endometrial proliferation and differentiation. This study was done to evaluate the effect of G-CSF in improving endometrial thickness and pregnancy rate in infertile patients undergoing stimulated IUI (intrauterine instillation) having thin endometrium.

Methods: This was a non-randomized clinical trial done among 40 infertile patients with thin endometrium (<7 mm) on the day of ovulation trigger in stimulated IUI patients. Study subjects were non randomly allocated into 2 groups. In group A 20 patients received intrauterine instillation of G-CSF (300 mcg/0.5 ml) via intrauterine catheter on triggering day and in group B another 20 patients received intrauterine instillation of 0.5 ml normal saline in the same procedure. After 48 hours endometrial thickness was measured in both groups. IUI was done on the same day. Pregnancy was detected by serum beta hCG level after 14 days of IUI.

Results: In both groups most of the respondents were aged between 30-34 years, 14 (70%) in group A and 11 (55%) in group B. The mean±SD of age 32.4±3.1 in group A, 32.2±3.4 in group B and P value was 0.9. In group A the minimum endometrial thickness on day of ovulation trigger was 4.4 mm and after 48 hours of treatment with G-CSF, it was found 6.3 mm. The maximum endometrial thickness recorded in group A was 6.9 mm and it also increased to 8.7 mm after G-CSF treatment and followed by in group B min thickness 4.8 mm increase to 5.2 mm and max thickness 6.8 mm increased to 8.7 mm. The mean±SD of ET (mm) on the day of ovarian trigger was 5.8±0.8 which increased to 7.4±0.8 in group A and followed by 5.9±0.6 to 7.1±0.9 in group B. ET mean change (mean±SD) for group A was 1.6±0.7 which was greater than the group B 1.3±0.8. Pregnancy rate was 2 (10%) in group A and 1(5%) in group B.

Conclusions: Mean increase in endometrial thickness and pregnancy rate was higher in G-CSF group than normal saline group, but the difference was not statistically significant.

Keywords: G-CSF, Endometrial thickness, Intrauterine insemination

INTRODUCTION

When the endometrium fosters the conversion of endometrial cells into decidual cells, encourages blastocyst invasion, and stimulates placenta growth for the continuation of a successful pregnancy, this is referred to as being receptive.[1] Endometrial thickness (ET) and endometrial pattern have been proven in numerous studies to be predictive indicators of endometrial receptivity. Precise and particular endometrial maturational development is essential for implantation.[2] ET has long been utilized as a predictive indicator for embryo transfers as a sign of the endometrium's receptivity.[3] For a pregnancy to be successful, the endometrium must be of sufficient thickness.[4] Endometrium with a thickness of less than 7 mm is frequently regarded as being less than ideal for transfer and having a lower likelihood of becoming pregnant. Therefore, having a thin endometrium is a very bad aspect that effects on an ongoing pregnancy.[4] ET in the secretory phase is typically between 7 and 14 mm, and it is a critical component of successful implantation.[2,5] Other studies have demonstrated that endometrial thickness less than 6 mm reduces the likelihood of conception.[6,7] It is highly challenging to increase endometrial development in people with thin endometrium. As a result, there is a reduction in these patients' odds of becoming pregnant. There are several ways to increase endometrial thickness. Some conventional approaches include taking sildenafil vaginally, pentoxifylline, tocopherol, arginine, and low doses of aspirin. G-CSF is one of the more recent newer approaches that has been developed because many times administering these agents is ineffectual or some methods require longer duration of therapy for effective increase in endometrial thickness. Implantation is greatly aided by the endometrium's immunological mechanisms.[8] G-CSF induces neutrophil recruitment and vascular endothelial growth factor release, which results in vascular remodelling, endometrial growth, uterine gland transformation, and the growth of new blood vessels, all of which improve uterine blood supply and result in endometrial decidualization and endometrial thickening. G-CSF is a type of colony stimulating factor that is produced by several immune cells, macrophages, and endothelium in the body. It is crucial for the growth of follicles, ovulation and embryo implantation.[9] G-CSF has been shown to be a novel treatment for infertile patients with unresponsive thin endometrium by Gleicher et al.[10]

Objective of the study

The objective of this study was to evaluate the effect of G-CSF in improving ET and pregnancy rate in infertile patients undergoing stimulated IUI having thin endometrium.

METHODS

This was a hospital based prospective study; carried out at the department of reproductive endocrinology and infertility for one year duration (September 2021 to August 2022) on 40 infertile patients attending outpatient department for treatment. For statistical analysis, SPSS version 20 was used as a statistical tool.

Inclusion criteria

All infertile women of age group 18 to 40 years having ET less than 7 mm when size of dominant follicle was more than 18 mm in stimulated IUI cycle were included.

Exclusion criteria

Patients with history of endocrine disorders, systemic diseases, severe endometriosis, congenital or acquired uterine anomaly were excluded from the study. Patient with intrauterine adhesions or polyps or sub mucous fibroids were excluded from this study. Patients with contraindication for use of granulocyte colony stimulating factor were excluded.

On the basis of pre-planned and pretested clinical examinations and investigations, infertile women who will undergo IUI cycles for treatment were selected after counselling and informed written consent. All 40 patients were split into two groups. Intrauterine instillation of G-CSF (300 mcg/0.5 ml) was administered in group A, whereas normal saline was administered in group B. Ovulation induction was administered with tablet letrozole 5 mg daily from day 2 to day 6 and recombinant FSH Gonal F 75 mg was given on day 5 and day 7. From day 8, there was a continuous TVS evaluation for endometrial thickness and folliculometry. Patients were chosen for intrauterine G-CSF instillation or saline installation if their ET was less than 7 mm on the trigger day (when follicular size was more than 18 mm). The content of G-CSF was aspirated into insulin syringe and instilled into uterine cavity with soft IUI catheter. Normal saline was also instilled in the same procedure. Triggering was done with chorionic gonadotropin injection Pregnyl 5000 IU in both groups. Two groups' endometrial thickness was once again measured by TVS using the same instrument 48 hours after instillation. IUI was then performed on the same day. 14 days after IUI serum beta HCG was measured for pregnancy.

RESULTS

Table 1 shows the age distribution of the respondents. In group A, most of the respondents 14 (70%) were aged between 30-34 years and followed by 2 (10%) were 25-29 years, 5 (20%) were 35-39 years where in the group B most of the respondents 11 (55%) were aged between 30-34 years and followed by 4 (20%) were 25-29 years, 5 (25%) were 35-39 years. The mean±SD of age 32.4±3.1 in group A, 32.2±3.4 in group B and p value was 0.9.

Table 2 shows the baseline characteristics of the respondents. The mean±SD of BMI for group A was 24.1±2.4 and for group B was 24.7±2.3 and followed by

the serum FSH (µIU/ml) (mean±SD) in both groups were 8±2.8 and 6.9±1.8, AMH (ng/ml) (mean±SD) was 3.6±1.5 and 4.1±1.2.

Figure 1 showed the type of infertility of the respondents. In group A 5 (25%) patients were found in primary and 15 (75%) were secondary where in group B 7 (35%) were primary and 13 (65%) secondary.

Table 3 displayed the patients' average endometrial thickness. In group A the minimum value of endometrial thickness on day of ovarian trigger was 4.4 mm and after 48 hours treatment with G-CSF, it was found 6.3 mm. The maximum ET recorded in the study patients on the day of ovarian trigger was 6.9 mm and it also increased to 8.7 mm after G-CSF treatment and followed by group B min thickness 4.8 mm increase to 5.2 and max thickness 6.8 increase to 8.7. The mean±SD of ET (mm) on the day of ovarian trigger was 5.8±0.8 which increased to 7.4±0.8 in group A and followed by 5.9±0.6 to 7.1±0.9 in group B. ET mean change (mean±SD) for group A was 1.6±0.7 which was greater than the group B.

Table 1: Age distribution of the respondents.

Age distribution (years)	Group A		Group B		P value
	N	Percentage (%)	N	Percentage (%)	
25-29	2	10.0	4	20.0	0.9
30-34	14	70.0	11	55.0	
35-39	4	20.0	5	25.0	
Mean±SD	32.4±3.1		32.2±3.4		

Table 2: Demographic and baseline characteristics of the respondents.

Demographic and baseline characteristics	Group A	Group B	P value
	Mean±SD	Mean±SD	
BMI (mean±SD) kg/m^2	24.1±2.4	24.7±2.3	0.3
Serum FSH (µIU/ml)	8±2.8	6.9±1.8	0.2
AMH (ng/ml)	3.6±1.5	4.1±1.2	0.4

Table 3: Comparison of ET.

Comparison of ET, mm	Group A			Group B			P value
	Min thickness (mm)	Max thickness (mm)	Mean± SD	Min thickness (mm)	Max thickness (mm)	Mean± SD	
ET (mm) on the day of ovarian trigger	4.4	6.9	5.8±0.8	4.8	6.8	5.9±0.6	0.4
ET after 48 hours	6.3	8.7	7.4±0.8	5.2	8.7	7.1±0.9	0.1
ET mean change		1.6±0.7			1.3±0.8		0.1

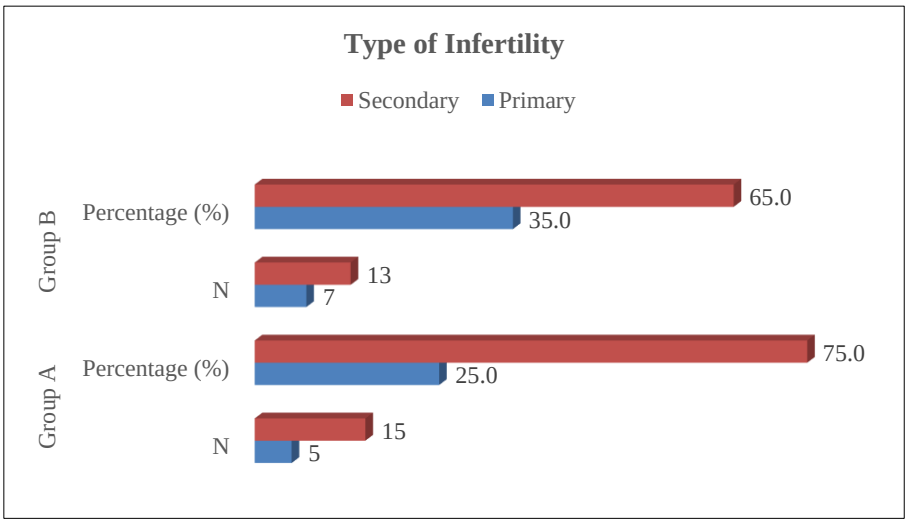

Figure 1: Type of infertility.

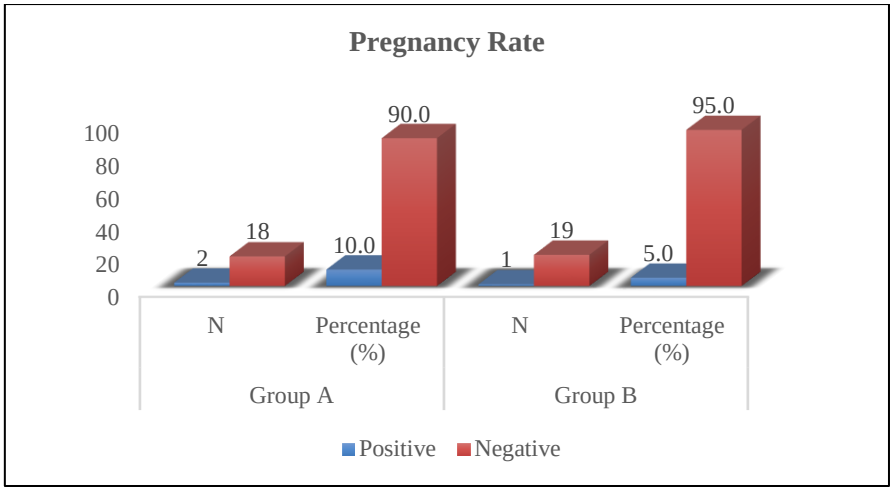

Figure 2: Pregnancy rate.

Figure 2 explained the pregnancy rate of the respondents. The pregnancy rate in group A (who received intrauterine instillation of G-CSF 300 mcg/0.5 ml) was 2 (10%) where in group B (who received intrauterine instillation of 0.5 ml normal saline) was 1 (5%).

DISCUSSION

After intrauterine instillation of G-CSF 300 mcg/0.5 ml, endometrial thickness significantly improved in infertile women with thin endometrium, similar to what was observed in Gleicher et al and Mishra et al, Aleyasin et al, Shah et al, Xu et al, Kim et al, Tehraninejad et al and Kunicki et al study.[6,11-17] In our study we found that the average endometrial thickness of group A was 5.8±0.8 mm on the day of ovulation trigger, which was increased to 7.4±0.8 mm after 48 hours of intrauterine instillation of G-CSF (300 mcg/0.5 ml) via intrauterine catheter. The mean difference was 1.6±0.7 mm. In group B the baseline ET was 5.9±0.6 mm on the day of ovulation trigger which was increased to 7.1±0.9 mm after 48 hours of intrauterine instillation of 0.5 ml normal saline in the same procedure. The mean difference was 1.3±0.8 mm. The mean difference of ET change between the two groups was statistically insignificant ($p>0.05$). In all 5 cases, Gleicher et al discovered an increase in endometrial thickness >7 mm.[23] In a different investigation, Gleicher et al showed a notable rise in ET of 2.9±2 mm.[6,21] ET increased by 1.68±1.05 mm according to a study by Kunicki et al and Kim et al while ET increased by 2.5±1.2 mm.[12,14] After 4 and 21 days of G-CSF infusion, there was an average increase in ET of 2.5 mm in the Shah et al study.[16] According to a study by Xu et al the rise in ET was 2.4±1.4 mm.[13] According to Tehraninejad et al the rise in ET of 3.5±0.88 mm was a considerable amount.[15] In a recent meta-analysis, Li et al found that G-CSF perfusion might significantly improve endometrial thickness.[18] However, in some trials, such as the Eftekhar et al study, there was no improvement in ET after intrauterine G-CSF instillation.[19] They came to the conclusion that the use of G-CSF was ineffective in increasing the endometrium thickness among infertile women undergoing IVF because the difference in ET between the study and control group was similar in the studies by Aleyasin et al and Barad et al as well.[17,20] Only two of the 20 patients in group A of our study (10%) were pregnant after utilizing G-CSF on the day of the ovarian trigger and only one of the 20 patients in group B (5%) were pregnant after receiving normal saline. This was quite comparable to the studies by Mishra et al (15.1% pregnancy rate) and Kunicki et al (18.9% pregnancy rate).[11,12] In studies by Gleicher et al (19.1% pregnancy rate), Xu et al (48.1% pregnancy rate), Kim et al (29.4% in patients with poor endometrium), Tehraninejad et al (20% pregnancy rate), Shah et al (37% in thin endometrium), and Aleyasin et al.18 (44.6% pregnancy rate).[6,13-17] G-CSF significantly increased ET, clinical pregnancy rate, and decreased cycle cancellation rate as compared to control group in IVF cycles, according to Xie et al in a meta-analysis which included 11 trials.[21] However, there was no change in the implantation and pregnancy rates between the study and control group in Barad et al and Li et al study.[18,10] In contrast to all prior trials, we used G-CSF in patients with thin endometrium on the trigger day of IUI cycles rather than IVF cycles, and there was no appreciable difference in the proportion of pregnancies in the two groups.

CONCLUSION

Even today, assisted reproductive technologies face difficulties in promoting endometrial development in women with thin endometrium. It is safe and likely beneficial to increase ET by injecting G-CSF into the uterine cavity. We discovered that in women with a thin endometrium on the day of the ovulation trigger, the average ET increased following G-CSF intrauterine instillation. Additionally, it might, to a certain extent, improved IUI cycle pregnancy rates. Although the

difference between the G-CSF group and the normal saline group in this study was not statistically significant.

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REFERENCES

1. Zenclussen AC, Hämmerling GJ. Cellular regulation of the uterine microenvironment that enables embryo implantation. Front Immunol. 2015;6:321.
2. Senturk LM, Erel CT. Thin endometrium in assisted reproductive technology. Cur Opin Obstet Gynecol. 2008;20:221-8.
3. Jimenez PT, Schon SB, Odem RR, Ratts VS, Jungheim ES. A retrospective cross-sectional study: fresh cycle endometrial thickness is a sensitive predictor of inadequate endometrial thickness in frozen embryo transfer cycles. Reprod Biol Endocrinol. 2013;11:1-5.
4. Check JH, Nowroozi K, Choe J, Dietterich C. Influence of endometrial thickness and echo patterns on pregnancy rates during in vitro fertilization. Fertil
5. Loke YW, King A, Burrows TD. Decidua in human implantation. Hum Reprod. 1995;10(2):14-21.
6. Gleicher N, Kim A, Michaeli T, Lee HJ, Shohat-Tal A, Lazzaroni E, et al. 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.
7. 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.
8. Sharkey A. Cytokines and implantation. Rev Reprod. 1998;3(1):52-61.
9. Yanagi S, Makinoda R, Fujii S, Miyazaki S, Fujita H, Tomizawa K, et al. Cyclic changes of granulocyte colony-stimulating factor (GCSF) mRNA in the human follicle during the normal menstrual cycle and immunolocalization of G-CSF protein. Hum Reprod. 2002;17(12):3046-52.
10. Gleicher N, Vidali A, Barad DH. Successful treatment of unresponsive thin endometrium. Fertil Steril. 2011;95(6):2123.
11. Mishra V, Choudhary S, Sharma U, Aggarwal R, Agarwal R, Gandhi K, et al. Effects of granulocyte colony-stimulating factor (GCSF) on persistent thin endometrium in frozen embryo transfer (FET) cycles. J Obstet Gynaecol India. 2016;66(1):407-11.
12. Kunicki M, Łukaszuk K, Liss J, Skowrońska P. Granulocyte colony stimulating factor treatment of resistant thin endometrium in women with frozen-thawed blastocyst transfer. J Obstetr Gynecol Syst Biol Reprod Med. 2017;63(1):49-57.
13. 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):349-58.
14. 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):183.
15. Tehraninejad E, Enieh MD, Davari F, Asadi E, Kamali K, Koorosh MD, et al. G-CSF intrauterine for thin endometrium, and pregnancy outcome. J Fam Reprod Health. 2015;9(3):107-12.
16. 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):100-6.
17. Aleyasin A, Abediasl Z, Nazari A, Sheikh M. Granulocyte colony stimulating factor in repeated IVF failure, a randomized trial. Reproduction. 2016;151(6):637-42.
18. 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):381-5.
19. Eftekhar M, Hosseinisadat R, Baradaran R, Naghshineh E. Effect of granulocyte colony stimulating factor (G-CSF) on IVF outcomes in infertile women: an RCT. Int J Reprod Biomed (Yazd). 2016;14(5):341-6.
20. Barad DH, Yu Y, Kushnir VA, Shohat-Tal A, Lazzaroni E. A randomized clinical trial of endometrial perfusion with granulocyte colony stimulating factor in in vitro fertilization cycles: impact on endometrial thickness and clinical pregnancy rates. Fertil Steril. 2014;101(3):710-5.
21. Xie Y, Zhang T, Tian Z, Zhang J, Wang W, Zhang H, et al. 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).

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